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PARASITES OF THE FAMILY THEILERIDAE OF THE AFRICAN BUFFALO OCCURRING IN EAST AFRICA

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INTRODUCTION

Parasites of the Family Theileridae are ubiquitous, occurring in Europe, Africa, Asia, Australia and North America. They have been reported from a wide variety of animals including mammals as diverse as the field mouse and the reindeer. Two genera are included in the Family, Theileria and Cytauxzoon. Members of the genus Theileria appear to be relatively harmless and, in most cases, they live in harmony with their hosts; the three known species of Cytauxzoon caused the death of the antelopes in which they were discovered.

In Africa south of the Sahara two species of Theileria commonly infect cattle. The first, Theileria parva, is highly pathogenic and is the cause of the notorious East Coast fever, whilst the second species, Theileria mutans, is usually benign, only occasionally being responsible for an illness known as Tzaneen Disease. An excellent general review of the African Theileriases of domestic animals has been given by Henning (1956).

Parasites similar to the intra-erythrocytic piroplasms of T. parva and T. mutans can be found in the blood of most wild ruminants in Africa but very little is known about them. They all look very much the same and virtually nothing is known of their pathogenicity for their wild hosts, or, more important, for domestic animals.

Farmers and veterinarians in Africa have long suspected that some of the wild animals harboured East Coast fever. Particular suspicion has always centred around the African buffalo (Syncerus caffer) and this account is concerned solely with theilerial parasites of this animal.

THE CLASSIFICATION OF THE PIROPLASMS

The classification of the Sub-order Piroplasmidea is constantly being modified. It is not intended to add further to the present confusion but the expression of somewhat conservative views may serve to clarify the situation and will, at least, define the present attitude of the writer.

The whole history of the subject has been admirably reviewed by Neitz and Jansen (1956) and by Neitz (1956) and some changes were proposed by these authors. Barnett and Brocklesby (1959) have already stated, without giving reasons, that they do not accept the revised classification and Mackerras (1959) and Tsur, Hadani and Pipano (1960) have also rejected it. Support for the new nomenclature has come from F.A.O. Report 1958/24, Haiba (1958), Seneviratna and Kumaraswamy (1960) and, with qualifications concerning the higher taxa, from Levine (1961) and Shortt (1962).

Neitz and Jansen (1956) created a new Sub-order which they called "Leucosporidea" to include all the parasites previously known as Theileria and left only the Family Babesidae in the old Sub-order Piroplasmidea. The basis of this new separation was the fact that the Babesidae do not undergo cycles of pre-erythrocytic or exo-erythrocytic schizogony. However, it is possible that such a phase of development does exist and the fact that it has not yet been discovered is probably due more to the lack of research than to the lack of such a cycle. The recent description of Nuttalia danii (Tsur et al., 1960), which includes an account of exo-erythrocytic schizonts reminiscent of those of Theileria, emphasizes the close relationship between the

Families Theileridae and Babesidae. It is felt, therefore, that until extensive study of arthropod-induced infections with species of Babesia has failed to demonstrate pre-erythrocytic or exo-erythrocytic schizogony, or the lack of a tissue cycle of development is proved in some other way, the creation of the Sub-order Leucosporidea cannot be accepted.

The proposed Sub-order Leucosporidea was divided by Neitz and Jansen (1956) into two Families, the old Theileridae and the newly proposed Gonderidae. The former was to include only one genus and one species, Theileria parva, whilst the new Family Gonderidae was to include all the other parasites previously known as Theileria, such as T. mutans and T. annulata, under the revived generic name of Gonderia, together with two parasites of antelopes with the generic name of Cytauxzoon. The reason for this division was stated to be the fact that T. parva was the only member of the Sub-order in which intra-erythrocytic multiplication did not occur; in all the others multiplication was supposed to occur within the red blood cells. It seems, however, that the direct evidence that this is the truth is remarkably slight.

Nuttall, Fantham and Porter (1909) spent much time observing living piroplasms of T. parva within erythrocytes. In no case could they satisfy themselves that intra-corpuscular parasites underwent multiplication. What appeared to be divisions were, however, repeatedly seen. The parasite assumed a double pyriform shape, the swollen portions remaining connected by a thin strand of protoplasm. Such forms were observed for several hours but no further change took place. They concluded with the following statement:-

"We have not obtained any conclusive evidence that the parasites multiply within the infected corpuscles, but at times appearances were observed suggesting this possibility."

Nuttall and Fantham (1910) studied stained preparations and again were not able to convince themselves whether multiplication did or did not take place. They provided some illustrations which strongly suggested multiplication (Figs. 17-21, 28, 31, 34, 45-48).

Nuttall (1913) in summarising the earlier work stated:-

"We have never observed multiplication of the living parasites in the corpuscles, but we have seen them in a few rare instances escape from the corpuscle into the plasma ..... The appearance of the chromatin in some parasites suggests that multiplication may occur within the infected corpuscles, some of which contain up to eight parasites. If, however, multiplication occurs within the corpuscles it must take place very slowly or we should have observed it in the living parasite."

Cowdry and Danks (1933) had quite a lot to say about the multiplication of piroplasms of T. parva and in their Diagram I (p. 37) illustrated 4 theoretical ways in which this might take place. They concluded that the evidence that division occurred was not satisfactory yet they thought that multiplication did take place in one or other of the 4 ways that they illustrated. Both Nuttall and Fantham (1910) and Cowdry and Danks (1933) recorded the occurrence

of "cross forms" which, in other members of the Family are thought to represent dividing parasites (Dschunkowsky, 1952).

Wenyon (1926) states:-

> "Though they may sometimes be seen in pairs in the red cells, or occasionally in the cross forms, it is doubtful if these represent division stages as they do in the case of B. mutans, the morphological resemblance to which may be very striking."

Reichenow (1940) quoted Gonder (1910) as saying that multiplication of T. parva within red cells took place only exceptionally. However, Reichenow himself took the view that, though the piroplasms grew a little, they did not divide.

The present Russian view is that intra-erythrocytic piroplasms of all Theileria species do not divide (Zolotarev, 1956). Schaeffler (1962) records the occurrence of division forms in stained blood films of T. parva sent to him from East Africa.

It is plain from the references that have been given that the conclusion drawn by Tsur et al. (1960) that

> "It has not been finally established whether or not multiplication takes place in the erythrocytes"

is correct. In view of this the revival of the genus Gonderia cannot at present be accepted.

Admittedly T. parva differs from other members of the Sub-order in several respects, for example it is comparatively difficult to transmit T. parva by the

inoculation of blood. However, T. lawrencei has not yet been successfully transmitted by this method. Recovery from infection with T. parva is said to result in a state of sterile immunity whilst recovery from infections with the other members of the Sub-order usually results in the state of premunity. However, the work of Barnett (1956) has shown that cattle recovered from East Coast fever may sometimes become carriers of T. parva. He was able to transmit the organism by blood inoculation from an ox that had been mechanically infected six months previously and maintain the parasite by passage through ticks. Daly (1960) has expressed the belief that relapses may occur in cattle recovered from East Coast fever. He stated:-

> "The fact that our research workers, under laboratory conditions, have not succeeded in breaking down the immunity in East Coast fever does not convince me that this does not take place under natural conditions."

Adamson (1955), when discussing an outbreak of East Coast fever in Rhodesia after nearly seven years freedom from the disease, concluded:-

> "It can only be assumed that the sterile immunity previously ascribed to this disease does not exist under field conditions."

These observations emphasise the close relationship between T. parva and the other members of the Sub-order. The fact that a strong cross-immunity exists between

T. parva and T. lawrencei further confirms this. Therefore until critical experiments produce conclusive evidence that T. parva is the only member of the Sub-order in which intra-erythrocytic multiplication does not occur, the Family Gonderidae and hence the genus Gonderia, cannot be valid. I am not convinced that even if this fact was demonstrated it would constitute sufficient reason for the separation.

It may well be that the classification proposed by Neitz and Jansen is the correct interpretation of these related parasites but the following three statements should be proved to be true before it is considered for acceptance.

1. Members of the Family Babesidae do not undergo pre-erythrocytic or exo-erythrocytic schizogony. [Recent work by Hoyte (1961) strongly suggests that B. bigemina does not have a tissue cycle].

2. Piroplasms of "Gonderia" and Cytauxzoon species do multiply within red cells.

3. Piroplasms of T. parva do not multiply within red cells.

The classification which it is suggested should be retained and which is used throughout this account is as follows:-

Sub-order Piroplasmidea Wenyon, 1926

Family Theileridae Du Toit, 1918

Genus Theileria Bettencourt, Franca & Borges, 1907.

Genus Cytauxzoon Neitz & Thomas, 1948.

Family Babesidae Poche, 1913.

Genus Babesia Starcovici, 1893 [and possibly some others such as Nuttalia and Echinozoon].

Since the above was written Neitz, at the Second Meeting of the FAO/OIE Expert Panel on Tick-Borne Diseases of Livestock, held in Cairo from 3rd - 10th December, 1962, reported as follows:-

> "In a series of critical experiments it has been established that Theileria parva intra-erythrocytic parasites can maintain themselves in splenectomised cattle in the complete absence of schizonts.....
> The genus Gonderia and the family Gonderidae

REVIEW OF THE LITERATURE

The African buffalo (Syncerus caffer) has for many years been suspected of being implicated in the epizootiology of East Coast fever or a similar disease. Various investigators and field workers in different parts of Africa have made observations but only in South Africa and Kenya has it definitely been proved that the buffalo can act as a reservoir of an East Coast fever-like disease.

The pertinent literature will be discussed in two parts:

a) The evidence that the buffalo may act as a reservoir of classical East Coast fever caused by Theileria parva.

b) The evidence that the buffalo acts as a reservoir of an unusual theilerial parasite, Theileria lawrencei. This will be discussed on a regional basis.

a) The Buffalo and Theileria parva

One of the first suggestions that the buffalo played a part in the spread of East Coast fever was that of Richardson (1930) who argued that the syndrome known as East Coast fever was due to a dual infection with a virus and T. mutans and that the two organisms acted in synergism. He thought that the buffalo was a carrier of the virus but not of T. mutans and supported this theory by reporting that when cattle, free of T. mutans, were exposed on buffalo grazings in Uganda, most of them suffered a mild disease and theilerial schizonts were seen only in one fatal case. He assumed that the majority of the cattle became infected

with the buffalo virus but, since the cattle were not carriers of T. mutans, East Coast fever did not develop. [It is possible that Richardson had in fact produced an outbreak of disease due to a comparatively mild strain of T. lawrencei].

In 1943 Lewis reported some interesting experiments with four buffaloes. Three of these were infested with R. appendiculatus ticks infected with T. parva but only one became infected and underwent a mild febrile reaction. The temperature never exceeded 102.0°F. and schizonts were seen for only 2 days in smears of the sub-parotid lymph node. Intra-erythrocytic piroplasms were seen in very small numbers and uninfected nymphae of R. appendiculatus which fed on the buffalo at the time of its fever subsequently transmitted East Coast fever when, after moulting to adults, they were allowed to feed on 3 steers. Ticks of the same batch failed to transmit the disease to the fourth buffalo. Lewis concluded that the African buffalo was sometimes susceptible to East Coast fever and might be responsible for sporadic outbreaks of the disease.

In a comprehensive review of the theileriases, Neitz (1957) stated that the role played by the African buffalo as a reservoir of East Coast fever had not been satisfactorily determined but that the work of Lewis (1943) suggested that it could be maintained under natural conditions in the complete absence of cattle. He further stated that:-

> "... in enzootic areas buffalo calves sooner or later contract East Coast fever and thus act as reservoirs".

No evidence was given to support this statement.

Several investigators have examined blood and tissue smears from apparently healthy buffaloes shot for sporting or control purposes. Viljoen (1924) recalled a conversation with Sir Arnold Theiler and believed that the latter had seen bodies like Koch's bodies in a smear from a buffalo. Daubney (1939) commented on circumstantial evidence connecting outbreaks of East Coast fever with the finding of Koch's bodies in eland and buffalo but gave no details. Walker (1932) infected a buffalo with T. mutans but no symptoms, other than slight icterus and fever, were elicited. He failed in attempts to infect the same buffalo with T. parva by exposure on an infected pasture and by allowing infected ticks to feed on its ears. Lawrence (1936) whilst investigating an "Undiagnosed disease of cattle" in Southern Rhodesia, a disease which later became known as "Theileriosis" and was eventually established as being due to T. lawrencei, became suspicious at that early date that the condition was in some way associated with buffaloes. In one buffalo spleen smear he saw rare theilerial schizonts; these are discussed later.

Nobody has yet succeeded in isolating typical East Coast fever directly from wild buffaloes. The available evidence, however, suggests that these animals do play some part in the epizootiology of the disease.

b) The Buffalo and Theileria lawrencei

Although the first observations on the disease known variously as Corridor Disease, Theileriosis, Specific Disease, Malignant Syncerine gonderiosis, Buffalo Disease, January Disease and Matusi, were made in Southern Rhodesia by Lawrence and others, a description of the work of Neitz and his associates in South Africa will be given first. This

was the first critical experimental work carried out on the condition and a knowledge of this will clarify the discussion of observations made in Rhodesia and elsewhere.

South Africa

The first mention of Corridor Disease in South Africa occurs in an interesting paper by Bigalke and Neitz (1950) who, whilst discussing the possible domestication of some wild ungulates, mentioned that observations had shown that the African buffalo was susceptible to Corridor Disease (Neitz, W. O., Adelaar, T. F. and Kluge, E., 1953. Field observations in Zululand. Not published. Onderstepoort Veterinary Laboratory). The name "Corridor Disease" was coined because the condition was first encountered in an area called "the Corridor", a stretch of country, 100 square miles in extent, lying between the Hluhluwe and Umfolozi Game Reserves in Zululand. The first full descriptions of the investigations in this area were given by Neitz, Canham and Kluge (1955) and Neitz (1955). The work is reviewed by Neitz (1957). The main interest of the work lies in two aspects. Firstly, the evidence that the African buffalo was implicated and, secondly, the evidence that the disease in cattle was different from East Coast fever.

The first suggestion that the disease differed from East Coast fever came when the affected herds of cattle were removed from the Corridor. The disease at once ceased to spread even though efficient tick vectors of T. parva were present on the new grazing areas. This suggested that the affected cattle did not infect ticks. Blood smears showed only a limited number of piroplasms, which were indistinguishable from T. mutans; they never exceeded 50/1000 R.B.C. There was thus no production of piroplasms such as occurs in East Coast fever when more than

600 piroplasms/1000 R.B.C. are commonly seen. An examination of smears of spleen, liver, lymph nodes and kidney revealed theilerial schizonts in small numbers. As a rule less than 5% of the lymphocytes were parasitised: in East Coast fever more than 80% of the lymphocytes were said to harbour schizonts. The schizonts in Corridor Disease appeared as round or oval bodies with 1 - 16, rarely more, chromatin granules. The size was stated to vary between 1 μ and 10 μ with an average of 5 μ but no indication was given of the number of schizonts measured to arrive at these round figures. The schizonts of T. mutans and T. parva were stated to vary between 1 μ and 15 μ with an average diameter of 8 μ; again no protocols have been published to support these figures. [At this point it is interesting to note that Barnett, Brocklesby and Vidler (1960) found that the average size of 1750 schizonts of T. parva was 4.8 μ].

Neitz et al. (1955) were able to transmit the disease to cattle with ticks collected from the vegetation in the Corridor but determined and extensive attempts to transmit the disease via ticks collected from infected cattle all failed.

Two buffalo calves were examined and one of these was shown by xenodiagnosis to be a carrier of the parasite. Two other buffalo calves were captured whilst incubating the disease and both of them died.

In view of the differences from T. parva exhibited by the causal parasite, Neitz (1955) felt justified in erecting a new species and named it Theileria lawrencei in honour of Dr. D. A. Lawrence who first investigated the disease caused by it in Southern Rhodesia in 1934. As mentioned earlier, Neitz and Jansen (1956) subsequently modified the classification of the Sub-order Piroplasmidea so that their new name for the parasite became Gonderia lawrencei. I have

already given my reasons for rejecting the taxonomy and therefore refer to the parasite as Theileria lawrencei Neitz, 1955.

At this point it is necessary to state that the Veterinary authorities in Southern Rhodesia had come to the conclusion, purely from epizootiological observations, that recovered cattle could act as reservoirs of the parasite. This led Neitz (1957) to decide that the Rhodesian parasite was different from Theileria (= Gonderia) lawrencei and he therefore created a new species and named the Rhodesian parasite Gonderia bovis (Neitz, 1957). However, whilst this paper was in press he (Neitz, 1958a, 1958b) succeeded in transmitting T. lawrencei from ox to ox by means of adult R. appendiculatus which had fed, as nymphae, on a splenectomised animal that had recovered from the infection. Neitz (1957), in a footnote, sank the newly created species and synonymised it with T. lawrencei. He concluded that cattle could become carriers of T. lawrencei but did not report any positive results using intact recovered animals.

Southern Rhodesia

The history of the introduction of East Coast fever into Rhodesia has been well described by Henning (1956). Suffice to say here that it resulted from the shipment of cattle from Tanganyika in 1901 and the disease eventually spread over the whole of the country where suitable vectors occurred. It was not until 1958 that Lawrence was able to state:

> "East Coast fever which has played such havoc with our cattle since the beginning of the century appears to have been successfully eliminated - the last outbreak, and that only a spasmodic one after six years of freedom,

As far as I have been able to discover no further outbreaks have been reported.

However, in 1934, Lawrence reported the occurrence of a new disease which came to be known as "Theileriosis" and which was later identified with Corridor Disease (T. lawrencei infection). It is with this infection that this account is chiefly concerned.

Hooper Sharpe (1934) gives a table (page 6) showing the annual incidence and mortality due to "African Coast Fever" (E.C.F.) from 1906-1933. Table I is an extension of this information to show how the condition "Theileriosis" waxed as the incidence of East Coast fever waned.

The obvious question stimulated by Table I is "What were the differences between East Coast fever and the condition that became known as Theileriosis?".

To try to answer this question reference must be made to the early reports of Lawrence and others.

Lawrence (1934, 1935), under the heading "Undiagnosed Disease of Cattle" discussed a heavy annual mortality of cattle in well defined areas and gave an account of the clinical symptoms:

> "Blood smear examination may fail to reveal any deviation from normal even on extensive search, or a very limited, or rarely extensive, infection with Th. mutans may be found. Gland smears are as a rule negative, but in some instances a few, or rarely even numerous, plasma (Koch's) bodies may be found."

Table I

Bovine theileriases in Southern Rhodesia

Year	Mortality due to East Coast Fever	Mortality due to "Theileriosis"
1935	124	?
1936	120	44 (146)
1937	128 (57)	1 (33)
1938	221 (5)	? (18)
1939	100 (26)	13 (15)
1940	428 (320)	27 (23)
1941	76 (71)	? (10)
1942	0	? (2)
1943	66 (2)	? (17)
1944	25 (2)	10 outbreaks
1945	About 50	19 outbreaks
1946	41	82
1947	Not consulted	Not consulted
1948	About 2	15 outbreaks
1949	0	153 (47)
1950	0	About 144 (29)
1951	0	176 (49)
1952	0	86 (37)
1953	0	559 (24)
1954	4 (4)	373 (18)
1955	0	885 (19)
1956	0	390 (52)
1957	0	601 (10)
1958	0	437 (17)
1959	0	274 (8)

Table I contd.

Note: 1) The bracketed figures are the number of slides positively diagnosed by the laboratory services.

2) Table I was constructed by reference to Adamson (1951, 1953, 1954, 1955), Christie (1952, 1956, 1957, 1959), Hooper Sharpe (1936, 1937, 1938), Huston (1947a, 1947b, 1947c, 1948, 1949, 1950), King (1947a, 1947b), Lawrence (1936, 1937, 1938, 1939, 1940, 1941, 1942, 1947a, 1947b, 1947c, 1947d, 1949, 1950, 1951, 1953, 1954, 1956a, 1958), Mackinnon (1952, 1954, 1956, 1957, 1958, 1959), Myhill (1939, 1940, 1941) and Nixon (1953).

He failed to transmit any disease by the inoculation of blood and lymph node material from the affected cattle into experimental sheep and cattle. It was found that mortality ceased abruptly if the affected herds of cattle were moved to 'clean' areas and also that if the affected cattle were then herded with susceptible animals the latter did not become infected. Lawrence was not at that time able to come to any satisfactory conclusions regarding the aetiology of the condition.

The next year, 1935, Lawrence (1936) mentioned that there was an association between the disease and the presence of buffaloes and he reported the finding of schizonts in a spleen smear from a buffalo:

> "This smear showed the presence of rare plasma bodies (Koch's Blue bodies) indistinguishable from those of East Coast fever, and more numerous atypical plasma bodies resembling the type most frequently found in positive smears from cattle affected with the undiagnosed disease."

This was the first intimation that the schizonts of the new parasite were different from those of T. parva. Lawrence thought that they more nearly resembled the schizonts of T. mutans which were:

> ".... in some subtle way different from the majority of those in East Coast fever."

He concluded that:

> ".... one would not be justified in assuming that the buffalo infection is associated with the disease of cattle."

The report for the next year, 1936, (Lawrence, 1937) included a detailed account of the investigations. He gave a description of the normal course of infection with T. parva and T. mutans and then pointed out how infection with the new parasite differed from them. The points of difference were (a) the extremely rapid course, (b) the absence of the usual post-mortem findings, (c) the abundance (sic) of Koch's bodies and (d) the almost if not complete absence of small piroplasms. The possibility that the disease was a peracute form of East Coast fever, causing the death of the animals before piroplasm production had commenced, was considered but later rejected since later cases, with a course of a fortnight, also had no infection of their erythrocytes. It was demonstrated that the disease was tick-borne by leaving half a group of susceptible cattle undipped. The main post-mortem differences were:- in 'theileriosis' there was no ulceration of the abomasum; the kidneys were normal; the liver was icteric; enteritis was most marked in the large intestine and there was marked hydrothorax.

> "Koch bodies could be demonstrated in spleen and gland smears, in some cases being as numerous as in a typical case of East Coast fever, but in other cases being rare and difficult to demonstrate. The vast majority of these bodies were distinctly of the agamogenous type .... as a rule not even a single small piroplasm could be found in spite of prolonged and careful search of perfect blood smears."

All attempts to transmit the disease by inoculations failed.

Many ticks were collected from the grass of the affected area but failed to transmit the disease to 2 cattle at the laboratory.

Lawrence at this stage concluded that the disease be "at least temporarily" regarded and controlled as East Coast fever.

In 1937, Lawrence (1938) challenged three of the cattle that had recovered from Theileriosis with ticks infected with the South African strain of T. parva. One of these died from East Coast fever, one died from East Coast fever complicated by a fractured femur and the third reacted and recovered. It was concluded that the parasite responsible for the disease could not have been T. parva, T. mutans or T. annulata.

In subsequent years, Lawrence (1939, 1940, 1941, 1942, 1947a-d) described further investigations. It was established that a cross-immunity did in fact exist between T. parva and the new parasite. Various chemotherapeutic agents were tried out but not with any significant success. These included formalin, mercurochrome, Uleron, sulphapyridine, calcium chloride and acaprin. Lawrence later tried Nivaquine, penicillin and Aureomycin and Christie (1957) also reported no success with Aureomycin and with Terramycin.

Lawrence (1956a) gave the first suggestion that differential diagnosis was becoming rather difficult:

> "... today there appears to be developing a closer resemblance between it [Theileriosis] and East Coast fever than formerly, in that macroscopic kidney lesions are sometimes evident, though not presenting the typical picture of an East Coast fever kidney, the Koch bodies are more frequent in smears, and the small piroplasms

> invading the erythrocytes, that were previously extremely difficult to detect in the majority of cases, are now found comparatively easily in most."

Further indications that the parasite was becoming more and more like T. parva can be deduced from remarks by Adamson (1951):

> "As bodies resembling Koch bodies which are found in African Coast fever occur in this disease.....",

Mackinnon (1952):

> "This disease, so closely resembling African Coast fever"

and Lawrence (1956b), whose remarks were reported as follows:

> "he had not been able to demonstrate erythrocytic forms and diagnosis of the disease (theileriosis and Corridor disease) was based on the presence of the small number of Koch's bodies and the absence of erythrocytic forms. In later investigations, however, the presence of erythrocytic forms, generally very rare, but occasionally in considerable numbers, was observed and Koch's bodies were sometimes as numerous as in E.C.F.".

It seems that the Theileria species responsible for Theileriosis in Rhodesia, which has been stated to be identical with Theileria lawrencei may be becoming more and more difficult to distinguish from T. parva.

Lastly, an interesting feature of the Rhodesian Theileriosis is the belief that recovered cattle can act as carriers of the causal parasite and therefore set up foci of infection when moved to farms where dipping is inadequate. This conclusion was not based on experiments but was deduced from the epizootiological observations of acute observers (Adamson, Hooper Sharpe, Huston, King, Lawrence, Mackinnon, Myhill and Nixon). The fact that the disease occurred in cattle in areas where no buffaloes existed lent support to this view. Indeed Neitz (1957) considered that the epizootiological evidence was strong enough to warrant the description of the causal parasite of Rhodesian Theileriosis as a new species.

It appears that the crucial experiment to decide whether cattle recovered from infection can become carriers of this parasite, xenodiagnosis using intact cattle, has not yet been done. It will, however, be recalled that Barnett (1956) reported successful demonstration of the carrier state in an animal mechanically infected with T. parva and that Daly (1950) and Adamson (1955) have both expressed the view that the concept of sterile immunity after recovery from East Coast fever may not apply under field conditions.

Nyasaland

Neitz (1957) expressed the opinion that the disease might also occur in Nyasaland since cattle spleen smears sent to him by S. G. Wilson in 1947 revealed a small number of schizonts indistinguishable from those of T. lawrencei. There was no suggestion of an association with African buffaloes.

Faulkner (1959, 1960) reported a disease from the Northern part of the country known locally as "matusi". The symptoms of the disease were typical of theileriasis and it was diagnosed as being due to G. bovis (= T. lawrencei).

Tanganyika

Milne (1956) described four cases of a disease which he thought might be distinct from East Coast fever. They were characterised by the fact that, whilst

> "numerous schizonts could be seen in blood, spleen and gland smears, erythrocytic forms were absent or only a few small bodies could be seen."

After some correspondence with the Southern Rhodesian authorities Milne cautiously concluded

> "It seems at least possible then that we were dealing with a similar condition to that found in Southern Rhodesia."

The Congo

Neitz (1957) believed that Corridor disease might occur in the Kisenyi area since in slides submitted to him by L. Bugyaki in 1955 bodies like T. lawrencei were present. R. appendiculatus nymphae that fed on the affected cattle failed to transmit the disease to susceptible cattle at Kisenyi or at Onderstepoort, South Africa.

Kenya

Barnett and Brocklesby (1959) described the isolation of a parasite they considered to be T. lawrencei from two areas of Kenya. They recovered the parasite from a wild buffalo, also by collecting ticks from cattle-free buffalo-infested grazings and by exposing cattle in such areas. Some of these observations will be extended in later discussions.

Since the discussion of the literature has necessarily been somewhat protracted, it might be helpful to present a brief summary.

Several observers have seen theilerial schizonts in buffalo material and Lewis (1943) demonstrated that the buffalo might act as a maintenance host of T. parva. Lawrence (1934 et sqq.) and others collected strong circumstantial evidence that an atypical theileriasis occurred in Southern Rhodesia, that the disease was associated with buffaloes, but that since recovered cattle may act as carriers of the causal parasite, the disease could be maintained in the absence of buffaloes. Neitz et al. (1955) and Neitz (1955) described the disease as Corridor Disease from Zululand and showed that the buffalo was the natural carrier of the causal parasite, which was named T. lawrencei. Infected cattle did not infect ticks but, oddly enough, after recovery and splenectomy, were able to do so. The parasite was differentiated from T. parva for the following reasons:

(1) Intra-erythrocytic piroplasms were not produced in infected cattle.

(2) Schizonts were smaller than those of T. parva and were produced in smaller numbers.

(3) Recovered cattle could become carriers of the parasite.

T. lawrencei has also been found in Kenya and there is some evidence that it occurs in the Congo, Tanganyika and Nyasaland.

MATERIALS AND METHODS

Laboratory Strain of Theileria parva

The main concern from which the work described in this thesis arose was to discover whether the Theileriae carried by the African buffalo were pathogenic for domestic cattle and, if so, whether they were related to Theileria parva or not. Therefore cattle, which either failed to react when attempts were made to infect them with buffalo-Theileria or which did react and recovered, were later challenged with our laboratory strain of T. parva. This strain is referred to as Theileria parva (Muguga); its behaviour is well known and it has been characterised in a number of different publications (Anon., 1951; Barnett, 1957, 1960; Barnett and Bailey, 1955a, 1955b, 1955c, 1955d, 1955e, 1958a, 1958b; Barnett, Brocklesby and Vidler, 1961; Brocklesby, 1962; Brocklesby, Barnett and Scott, 1961; Brocklesby and Vidler, 1961; Piercy, 1956). The parasite is highly pathogenic for "high-grade" cattle, causing a morbidity rate of 88% and a mortality rate, in infected cattle, of 96% when 10 adult Rhipicephalus appendiculatus are used to transmit the infection. The incubation period varies from 10 to 25 days, with an average of 14 days, and in fatal infections the febrile period averages 12 days (4 to 19 days). Both macroschizonts, microschizonts and intra-erythrocytic piroplasms are, in a typical case, produced in large numbers. Macroschizonts have been studied in great detail and were found to have an average size of 4.8μ (0.8 - 15.9μ). They contained from 1 to 85 nuclei, with an average of 8. In autopsy smears there were from 4 to 760 schizonts per thousand lymphocytes, with an average of 264. For further details of this strain reference should be made to the papers cited above.

The Cattle

The cattle used were of the type known locally as "high-grade". This term implies that they were animals of predominantly exotic type, such as Ayrshire or Friesian, which included a very low proportion of Zebu blood. They were purchased from farms from districts known to be free from East Coast fever and, as mentioned above, were highly susceptible to the disease, only 12% failing to become infected when infested with 10 infected ticks. When on experiment they were examined daily, their rectal temperature was taken, a thin blood film was prepared and smears were also prepared from any enlarged superficial lymph nodes. The experimental cattle were housed in specially designed tick-proof stalls that eliminated the risk of accidental infections.

Maintenance of the Parasites and Tick Vectors

The methods used for the maintenance of T. parva in cattle and in its tick vector have been fully described by Bailey (1960). Exactly similar methods were used for the maintenance of "T. lawrencei (Kenya)".

Histological and Microscopical Methods

Standard histological techniques were used throughout the study. Thin blood films were prepared from experimental cattle and buffaloes each day and daily smears were also made from enlarged superficial lymph nodes. These were dry-fixed with methyl alcohol and stained with 10% Giemsa for 30 minutes.

Tissue sections were stained by a variety of methods but the method most commonly used was the Giemsa-colophonium technique described by Shortt and Cooper (1948).

Sections of ticks were processed according to the methods elaborated in these laboratories by Martin, Barnett and Vidler (in press).

Drawings were made with the aid of a Leitz camera lucida or with a Wild "Zeichentubus". This latter apparatus had many advantages including the fact that it was designed to enable the microscopist, whilst drawing, to use a binocular microscope in the normal position; it was therefore very much less exhausting to use than a camera lucida.

Measures of the Size of Macroschizonts

An ocular micrometer, calibrated against a slide micrometer, was used in a system giving a magnification of x 800. In order to compensate for the irregular shapes of schizonts seen in tissue smears two measurements were made. First, the maximum diameter of the schizont was measured and this was called the major axis. Secondly the maximum diameter at right angles to the major axis was measured; this was called the minor axis. The arithmetic mean of these two measurements was used to express the size of the schizonts.

Counts of the Number of Nuclei within Macroschizonts

Generally at least 100 parasites were examined in each smear, all the parasites in successive fields of view being included until 100 had accumulated. In lightly parasitised tissues it was sometimes impossible to glean so many. This was a simpler procedure to carry out than measuring the size of schizonts and it was felt that if significant differences existed between the schizonts of different species, then this measurement would be the easiest to apply under field conditions.

Estimations of Parasitosis

The number of intracellular and extracellular parasites, both macroschizonts and microschizonts, seen whilst 500 or 1000 lymphocytes were counted, was noted and recorded as S. (= schizonts)/1000 L. (= lymphocytes) e.g. "264(22)/1000 L." means that the smear contained 264 schizonts, of which 22 were microschizonts, per 1000 lymphocytes. This method may not give a true measure of the rate of increase of the parasite or of the absolute degree of parasitosis since the number of lymphocytes changes during the course of the disease. It was considered, however, that the method was the best that could be devised and that it would at least reveal trends or gross differences between species or strains.

RESULTS

THE OCCURRENCE OF THEILERIAL PIROPLASMS IN WILD BUFFALOES

During the course of this work thin blood films have been prepared from 46 wild buffaloes shot on normal control work or for scientific purposes. In no less than 40 of these theilerial piroplasms were found. Forty-two of the buffaloes were shot in the Kigezi District of Uganda, one was shot on the northern slopes of Mt. Kenya, one was killed in the Mitoma area of Ankole, Uganda and two were shot at Mweiga, near Nyeri, Kenya. To these must be added the animals captured and given or loaned to us for the work described in the next two sections.

THE SUSCEPTIBILITY OF THE AFRICAN BUFFALO TO INFECTION WITH T. PARVA

In order to repeat the work of Lewis (1943) various buffaloes were obtained from different areas of East Africa and attempts were made to infect them with East Coast fever. Before this was done it was established whether or not the buffaloes were carrying theilerial piroplasms; this was done by the examination of blood films and by xenodiagnosis using cattle susceptible to East Coast fever as receptors.

The case of each buffalo will be described separately.

Buffalo Brutus

This male calf originated in the West Nile Province of Uganda and had been in captivity for several weeks before arriving at this laboratory. He was found to be carrying a theilerial piroplasm and attempts were made to identify it. Such attempts were not repeated in full with subsequent buffaloes as confidence in our methods of declaring a bovine calf to be free of T. mutans had waned since we had succeeded in transmitting a theilerial infection with ticks even though we were unable to find piroplasms in thin blood films. However, in this case a Hereford calf was obtained and after repeated and lengthy examinations of thin blood films had failed to reveal any haematozoa the calf was declared to be free of T. mutans. Blood from Buffalo Brutus was inoculated into the calf and, after an incubation period of 31 days, piroplasms tentatively identified as T. mutans appeared in blood films and persisted for many weeks.

Xenodiagnosis was applied at the same time. Ticks which fed on the buffalo, at the time that the blood was infective by inoculation, failed to transmit any infection to cattle even though large numbers were used. R. appendiculatus

and R. evertsi were used as shown in Table II. The recipient Hereford calves (Nos. 2408 and 2775) had also been declared to be free of T. mutans.

It was concluded that the theilerial parasite carried by Buffalo Brutus was T. mutans but that it was not able to infect R. appendiculatus or R. evertsi. The possibility remains that it was T. barnetti (see below).

Having demonstrated that Buffalo Brutus was not a carrier of T. lawrencei or T. parva he was infested with 50 R. appendiculatus infected with T. parva. After an incubation period of 11 days the buffalo underwent a mild febrile reaction which is shown in Fig. 1. The sub-parotid and prescapular lymph nodes became enlarged but schizonts were seen only in smears prepared from the former. Rare macroschizonts were seen and were never more frequent than 1/1000 L. Intra-erythrocytic piroplasms were present throughout the febrile period but did not increase.

Uninfected nymphae of R. appendiculatus were allowed to feed on the buffalo during and after the febrile reaction. They dropped engorged on the days indicated by arrows on Fig. 1. After moulting to adults these ticks were infested on the ears of cattle with the following results. Those ticks which dropped engorged on days 15, 17 and 94 failed to transmit East Coast fever to cattle. Those ticks which dropped engorged on days 19, 21 and 24 transmitted typical fatal East Coast fever to cattle.

Ten months later the buffalo was challenged with two strains of T. parva and was solidly immune. He was then challenged with T. lawrencei and underwent a mild reaction (Fig. 7).

Figure 1

Buffalo Brutus. *T. parva* infection.

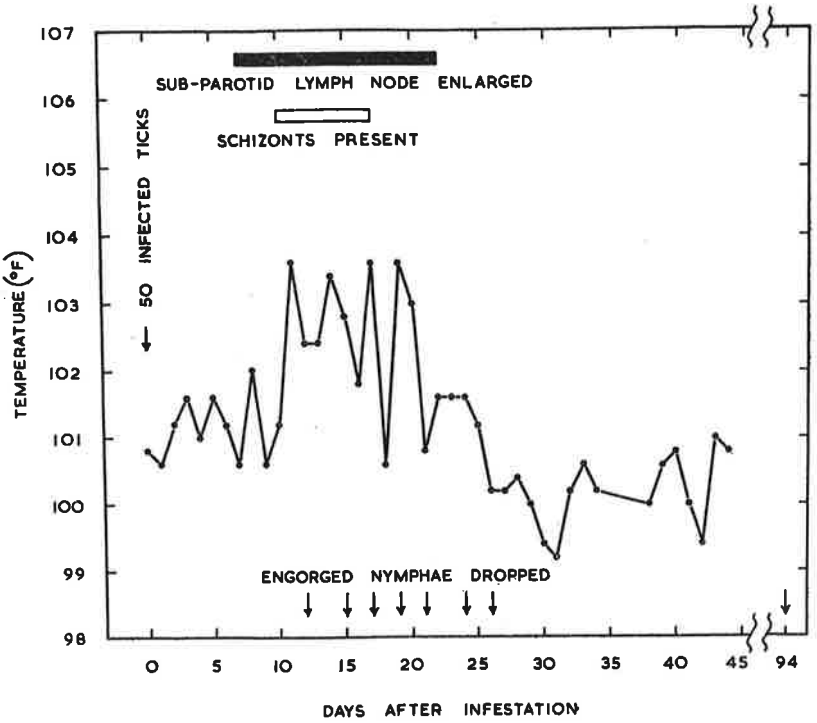

SUB-PAROTID LYMPH NODE ENLARGED
SCHIZONTS PRESENT
50 INFECTED TICKS
TEMPERATURE (°F)
107
106
105
104
103
102
101
100
99
98
ENGORGED NYMPHAE DROPPED
0
5
10
15
20
25
30
35
40
45
94
DAYS AFTER INFESTATION

Table II

Number & Species of tick infested	Number of recipient calf	Result
50 R. appendiculatus	2408	Blood negative for 14 weeks
(Calf Splenectomised)		" " " 6 weeks
186 R. appendiculatus	2408	" " " 12 weeks
24 R. appendiculatus	2408	" " " 12 weeks
24 R. evertsi	2775	" " " 13 weeks

Buffalo Venus

This animal was an adult female obtained from the Mara District of Kenya where she had been in captivity since calfhood. Small theilerial piroplasms were found in her red cells and xenodiagnosis was carried out, using nymphae of R. appendiculatus. After moulting to adults they were allowed to feed on cattle as follows:-

40 ticks on to Steer No. 5845. No reaction resulted. The steer was later challenged with T. parva and reacted and recovered.

40 ticks on to Steer No. 5837. No reaction resulted. The steer was later challenged with T. parva and died.

It was concluded that the buffalo was not carrying T. parva or T. lawrencei.

Ten adult R. appendiculatus infected with T. parva were then allowed to feed on Venus. The ticks fed well but the buffalo showed no evidence of infection and was therefore presumed to be immune to East Coast fever.

Buffalo Brenda

This animal was a young female obtained from the Queen Elizabeth Park in Uganda. Theilerial piroplasms were present in the red cells and xenodiagnosis was applied. Uninfected nymphae of R. appendiculatus were allowed to feed on the buffalo and after moulting to adults 50 of them fed on Steer No. 4202. No reaction resulted and the steer was later shown to be susceptible to T. parva. It was concluded that the buffalo was not a carrier of T. parva or T. lawrencei.

This buffalo then became virtually impossible to use as either ticks refused to attach or by constant ear wagging

the buffalo succeeded in removing the ear-bag containing the ticks. Buffaloes have very large ears and have constantly provided us with much difficulty in getting ticks to feed on their ears. As mentioned later all attempts to use Buffalo Brenda for the further passage of T. lawrencei failed. Similarly all attempts to feed T. parva-infected ticks were unsuccessful. As a last resort some ticks infected with T. parva were allowed to feed on a rabbit in order to "mature" the contained parasites. Their salivary glands were then dissected out, suspended in saline and injected into the buffalo. Twenty sets of salivary glands were injected subcutaneously in the ear and a further twenty were injected into a prescapular lymph node. The buffalo did not become infected and it was tentatively concluded that she was immune to East Coast fever.

Buffalo Rufus

Buffalo Rufus was a male calf captured in the South Kinangop area of Kenya and brought directly to the laboratory. Small theilerial piroplasms were present on the arrival of this animal and xenodiagnosis was applied in the usual way. Thirty ticks failed to transmit any infection to each of two steers, both of which were subsequently shown to be susceptible to infection with T. parva. It was concluded that the buffalo was not a carrier of T. parva or T. lawrencei.

The buffalo was then infested with 10 ticks infected with T. parva and, though no febrile reaction was produced, the sub-parotid lymph node local to the infested ear became enlarged on the 15th day and remained enlarged for 8 days (Fig. 2). Schizonts were present in smears for 2 days. There was no increase in the number of intra-erythrocytic piroplasms and no significant alteration in the total leucocyte count. Uninfected nymphae were placed on the animal on days

17 and 19 and dropped engorged on the days shown in Fig. 2. After moulting to adults they were allowed to feed on cattle as follows:

50 ticks of Days 23 and 25 on to Steer 7549. No reaction resulted. The steer was later challenged with T. parva and died.

50 ticks of Days 26, 27 and 28 on to Steer 7550. No reaction resulted. The steer was later challenged with T. parva and died.

It was concluded that the buffalo had undergone a mild infection with T. parva that was not capable of infecting ticks.

Buffalo Steve

This buffalo was a very young male obtained from the Mara District of Kenya and brought to the laboratory within a few days of capture. Three days after arrival it became infected with a parasite that is described later as Theileria barnetti n. sp. and which was not transmissible to cattle. After the schizogonous phase of T. barnetti had ceased the buffalo was infested with 10 ticks infected with T. parva. Although there was no febrile reaction (Fig. 3) the local sub-parotid lymph node became enlarged on the 10th day and remained so for 9 days. Smears of this node revealed extremely rare schizonts on days 11 and 12 only. Uninfected R. appendiculatus nymphae were placed on the buffalo on days 11 and 14 and dropped engorged on the days shown in Fig. 3. After moulting to adults they were used as follows:

50 ticks of Days 17 and 19 on to Steer 6155. No reaction resulted. The steer was later challenged with T. parva and died.

It was concluded that the buffalo had undergone a mild infection with T. parva that was not capable of infecting ticks.

Figure 2

Buffalo Rufus. *T. parva* infection

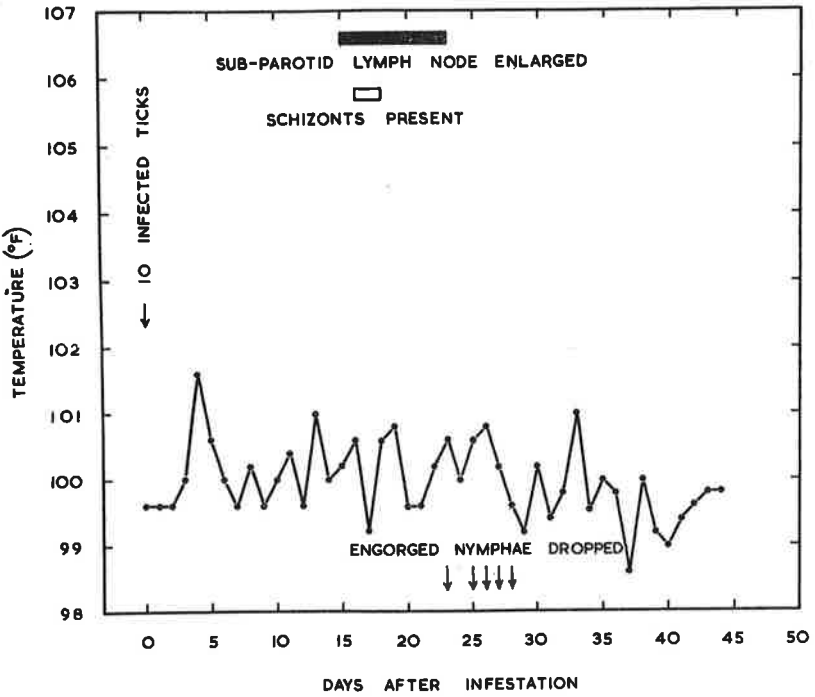

SUB-PAROTID LYMPH NODE ENLARGED
SCHIZONTS PRESENT
10 INFECTED TICKS
ENGORGED NYMPHAE DROPPED
TEMPERATURE (°F)
DAYS AFTER INFESTATION
107
106
105
104
103
102
101
100
99
98
0
5
10
15
20
25
30
35
40
45
50

Figure 3

Buffalo Steve. *T. parva* infection

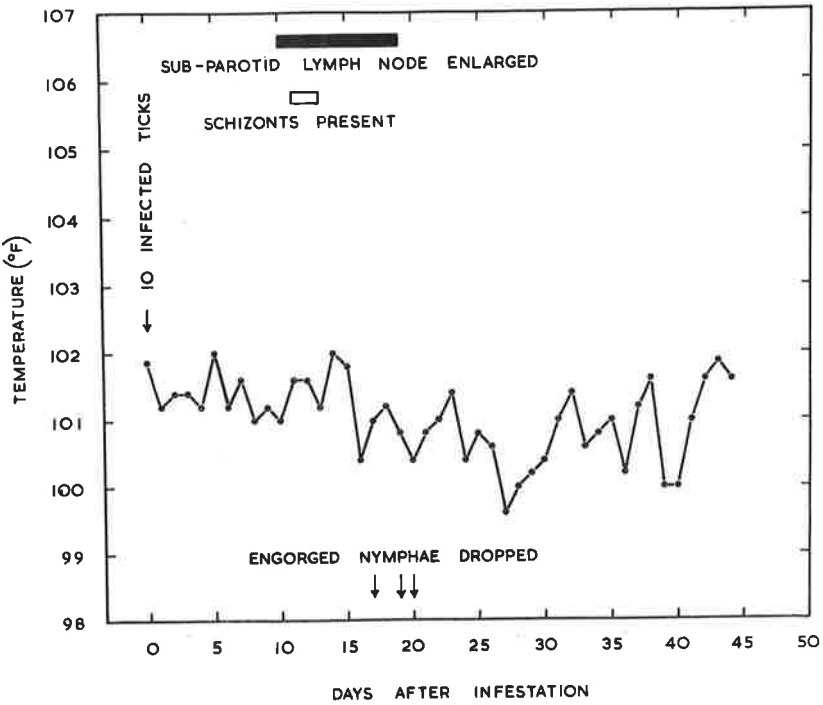
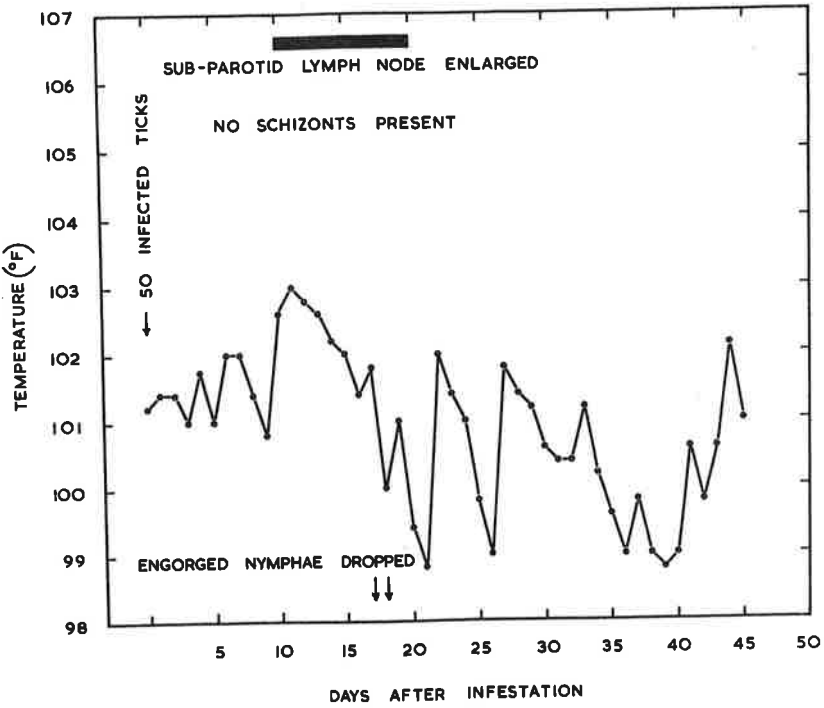

SUB-PAROTID LYMPH NODE ENLARGED
SCHIZONTS PRESENT
10 INFECTED TICKS
ENGORGED NYMPHAE DROPPED
TEMPERATURE (°F)
DAYS AFTER INFESTATION
107
106
105
104
103
102
101
100
99
98
0
5
10
15
20
25
30
35
40
45
50

Figure 4.

Buffalo Patrick. *T. parva* infection


SUB-PAROTID LYMPH NODE ENLARGED
NO SCHIZONTS PRESENT
50 INFECTED TICKS
ENGORGED NYMPHAE DROPPED
TEMPERATURE (°F)
DAYS AFTER INFESTATION
107
106
105
104
103
102
101
100
99
98
5
10
15
20
25
30
35
40
45
50

Buffalo Patrick

This animal, a male calf about 1 week old, was captured in the Mara District of Kenya and brought straight to the laboratory. He was lightly infected with a theilerial parasite which may have been T. mutans or T. barnetti (see below).

When the buffalo was 7½ months old, 50 ticks infected with T. parva were allowed to feed on it. After an incubation period of 10 days he underwent a mild febrile reaction lasting for 5 days and the local sub-parotid lymph node was moderately enlarged for 10 days (Fig. 4). Smears from this node and from the prescapular lymph node of the same side were carefully searched but, in spite of a very marked hypertrophy of lymphoid cells (which is characteristic of East Coast fever), no schizonts could be found. Since this buffalo was rather a weak individual the opportunity was taken to carry out a full haematological investigation. The erythrocyte count, haemoglobin and packed cell volume showed no alteration but the total leucocyte count fell from 12,000 to 6,000 cells/cu.mm. Uninfected nymphae were placed on the buffalo and dropped engorged on the days shown in Fig. 4. After moulting they were used as follows:-

80 ticks on to Steer No. 8081 and on to Steer No. 8137. No reactions resulted and both steers were later challenged with T. parva and died.

It was concluded that the buffalo had undergone a mild infection that was not capable of infecting ticks.

A summary of the available information on the susceptibility of the African buffalo to infection with T. parva is given in Table III.

Table III

The Susceptibility of the Buffalo to *T. parva*

Ref.	Number of Buffaloes	Methods of Infection	Result
Walker (1932)	1	Tick on ear and natural exposure	No reaction
Lewis (1943)	4	(i) Ticks on ear	No reaction
		(ii) Ticks on ear	No reaction
		(iii) Ticks on ear	Slight fever; rare schizonts; transmissible to cattle
		(iv) Ticks on ear (from 3rd buffalo)	No reaction
This paper	6	(i) Ticks on ear	No reaction
		(ii) Ticks on ear	No fever; L/N enlarged; rare parasites
		(iii) Ticks on ear	No fever; L/N enlarged; rare schizonts
		(iv) Ticks on ear	Slight fever; L/N enlarged; no parasites
		(v) Ticks on ear	Slight fever; L/N enlarged; rare schizonts; transmissible to cattle
		(vi) Injection of tick salivary glands	No reaction

L/N = Sub-parotid lymph node

From Table III it can be seen that a total of 11 buffaloes have been exposed to the disease; none of them died. Only 2 (18%) became sufficiently heavily infected to infect ticks that were feeding on them at the time. Two others became sufficiently infected for rare schizonts to be discovered and a third may have had a very mild infection causing only slight fever and lymph node enlargement.

THE ISOLATION OF "THEILERIA LAWRENCEI (KENYA)" FROM A WILD BUFFALO

The search for T. lawrencei in East Africa was carried out by three different methods. The first method involved the collection, by hand or by the employment of bait cattle, of ticks from the vegetation in areas known to be infested by buffaloes but where it could be stated with certainty that no cattle had grazed for a long period. The second method involved the exposure of cattle in such areas. The third method was more direct and consisted of making collections of engorged larval or nymphal ticks from wild buffaloes shot on normal control work. The results of this third method are described here.

Engorged larvae or nymphae are the only useful stages of the tick's life cycle for this work as theilerial parasites are only transmitted either by adults infected as nymphae or by nymphae infected as larvae; transovarial transmission does not take place. The engorged larvae and nymphae must be fully engorged and at the point of detachment. Such ticks are not very frequently found in useful numbers on wild animals; it is essential for the collector to be close behind the hunter and for him to start searching for ticks as soon as the animal is shot, for engorged ticks detach and walk away surprisingly quickly after the death of their host.

The cooperation of the Kenya Game Department was sought and most generously given for these studies. Mr. R. Havard, who was engaged in buffalo control work in the region of Nyeri, kindly allowed collections to be made from the animals he shot. During the period of our collaboration fourteen buffaloes were killed and 30 engorged nymphal ticks were found on one animal. This buffalo was a healthy adult female and was shot in the Nyeri Forest. The ticks were brought back to the laboratory

where they were allowed to moult to adults; they were then identified as Rhipicephalus appendiculatus.

Some of these were allowed to feed on cattle (see below) and others were placed on a captive buffalo, called Nero, who was kindly loaned to us by the Naivasha Experimental Station of the Kenya Veterinary Department.

Four captive buffaloes, including Buffalo Nero, were successfully infected with a theilerial parasite isolated from the buffalo shot in Nyeri Forest. This parasite will be referred to as "Theileria lawrencei (Kenya)". (The name is placed in inverted commas as evidence will be presented later that the parasite is in fact a strain of T. parva).

THE PASSAGE OF "THEILERIA LAWRENCEI (KENYA)" THROUGH SOME CAPTIVE BUFFALOES

The Buffalo Nero became infected with "T. lawrencei (Kenya)" and served as a source of infection for the subsequent passage of the organism through buffaloes and through cattle. Including Nero, four buffaloes have been infected and each case will be described separately.

Buffalo Nero

It was first necessary to discover whether or not the buffalo was a carrier of either T. parva or T. lawrencei. Many thin blood films were examined and extremely rare theilerial piroplasms were found. Xenodiagnosis was applied; on two occasions, at an interval of 5 months, large numbers of uninfected nymphae of R. appendiculatus were allowed to feed on the buffalo. These engorged and after moulting to adults were placed on cattle with the following results.

1st Batch. 50 ticks on to Steer No. 3191. No reaction resulted. The steer was later challenged with T. parva and died.

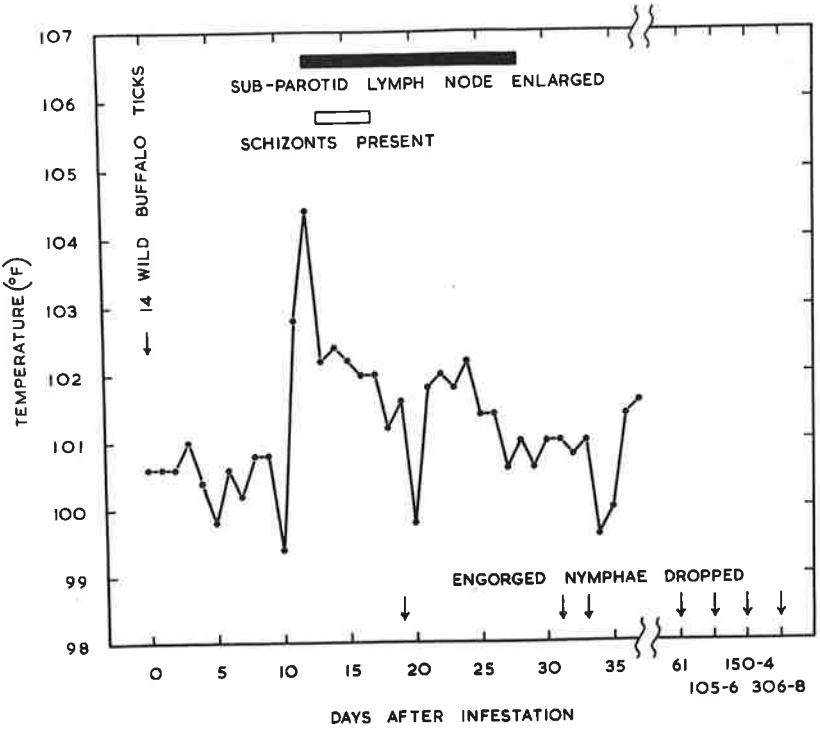
200 ticks on to Steer No. 2797. No reaction resulted. This steer was later challenged with T. parva and reacted and recovered.

2nd Batch. 60 ticks on to Steer No. 3672. No reaction resulted. The steer was later challenged with "T. lawrencei (Kenya)" and died.

It was concluded that the piroplasm carried by the buffalo was not T. parva or T. lawrencei.

Figure 5.

Buffalo Nero. "*T. lawrencei* (Kenya)" infection


107
106
105
104
103
102
101
100
99
98
TEMPERATURE (°F)
← 14 WILD BUFFALO TICKS
SUB-PAROTID LYMPH NODE ENLARGED
SCHIZONTS PRESENT
ENGORGED NYMPHAE DROPPED
0
5
10
15
20
25
30
35
61
150-4
105-6 306-8
DAYS AFTER INFESTATION

Fourteen of the ticks collected as engorged nymphae from the buffalo shot in Nyeri Forest were then allowed to feed on Buffalo Nero. The ticks fed well and after an incubation period of 11 days the buffalo underwent a fairly severe febrile reaction which lasted for approximately 7 days (Fig. 5). The sub-parotid, prescapular and prefemoral lymph nodes became enlarged and rare theilerial macroschizonts were discovered in smears. Microschizonts were never seen. Intra-erythrocytic piroplasms were produced in small numbers (up to 6/1000 R.B.C. on the 22nd day). There were no clinical symptoms other than the enlargement of the superficial lymph nodes. Uninfected nymphae of R. appendiculatus were placed on the buffalo on days 11, 13 and 24. These dropped engorged on the days indicated by the first three arrows in Fig. 5 and provided the source of further passages of the parasite in cattle and buffaloes.

Since schizonts were always rare it was not possible to carry out many measurements. However, after very long searches 100 schizonts were measured and they varied in size between 2.0μ and 15.2μ with an average size of 3.83μ. The numbers of nuclei were counted in 480 schizonts, taken from 9 different smears; the average number of nuclei was 5.15, with a range of 1-47 (Table IV).

Table IV

The Number of Nuclei within schizonts of "*T. lawrencei* (Kenya)" in Buffalo Nero

Number of Nuclei	Biopsy smears (480 schizonts)	
	Actual Figure	%
1	42	8.8 (0.4)
2	67	14.0 (3.4)
3	92	19.2 (8.0)
4	75	15.6 (12.3)
5	60	12.5 (11.9)
6	45	9.4 (11.2)
7	26	5.4 (9.3)
8	14	2.9 (7.4)
9	13	2.7 (6.0)
10	13	2.7 (5.1)
11	4	0.8 (4.2)
12	3	0.6 (3.9)
13	1	0.2 (2.8)
14	2	0.4 (2.3)
15	6	1.3 (1.8)
16	1	0.2 (1.5)
17	2	0.4 (1.2)
18	2	0.4 (1.0)
19	1	0.2 (0.9)
20	3	0.6 (0.9)
21	1	0.2 (0.6)
22	1	0.2 (0.6)
23	-	- (0.4)
24	-	- (0.4)
25	1	0.2 (0.3)
26+	5	1.0 (2.2)
Average	5.15 n/s	(7.97)
Range	1 - 47	(1-85)

The bracketed figures in the last column are the figures given by Barnett *et al.* (1961) for *T. parva* and are included in this

Schizonts in the Buffalo Nero were therefore considerably smaller than those of T. parva being, on average, 3.83μ in diameter compared with 4.8μ for T. parva and having an average of 5.15 nuclei per schizont (n/s) compared with 7.97 n/s in the case of T. parva. These features, combined with the general scarcity of piroplasms and the complete absence of microschizonts, led to the diagnosis of "T. lawrencei".

The buffalo made an uneventful recovery and attempts were made to discover whether or not it became a carrier of the parasite. Uninfected nymphal ticks were allowed to feed on the buffalo at intervals after recovery and dropped engorged on the days indicated by arrows on the right of Fig. 5. These ticks, after they had moulted to adults, were allowed to feed on cattle with the following results.

Ticks of Day 61.

10 on to Steer No. 3422. No reaction resulted. The steer was later challenged with T. parva and reacted and recovered.

20 on to Steer No. 4165. No reaction resulted. This steer was not challenged with T. parva.

Ticks of Days 105/106.

10 on to Steer No. 4101. No reaction resulted. The steer was later challenged with T. parva and died.

10 on to Steer No. 4121. No reaction resulted. The steer was later challenged with T. parva and died.

Ticks of Days 150-154.

35 on to Steer No. 4118. No reaction resulted. The steer was later challenged with T. parva and died.

Ticks of Days 105-106 and 150-154 (bulked)

40 on to Steer No. 4139. No reaction resulted. The steer was later challenged with T. parva and died.

Ticks of Days 304-308.

30 on to Steer No. 4532 and 30 on to Steer No. 4533. No reactions resulted. Both steers were later challenged with T. parva and died.

It was thus demonstrated that Buffalo Nero did not become a carrier of "T. lawrencei (Kenya)".

The buffalo was then challenged with T. parva and was completely refractory to infection.

Buffalo Ferdinand

This animal was a young male calf obtained from the Queen Elizabeth Park, Uganda. No theilerial piroplasms were discovered in blood films. Xenodiagnosis was applied and uninfected nymphal ticks were allowed to feed on the buffalo: after they had moulted to adults fifty of them were placed on Steer No. 4204 and no reaction resulted. The steer was later shown to be susceptible to T. parva. It was concluded that the buffalo was not a carrier of T. parva or T. lawrencei.

Ten adult ticks that had dropped as engorged nymphae from Buffalo Nero on day 31 (Fig. 5) were allowed to feed on Buffalo Ferdinand. The ticks fed well and after an incubation period of 15 days the buffalo underwent a severe febrile reaction and died on the 36th day. The superficial lymph nodes became enlarged and smears of the sub-parotid and prescapular nodes revealed an intense cellular hypertrophy and the presence of macroschizonts and microschizonts. Intra-erythrocytic piroplasms were produced in small numbers (up to 30/1000 R.B.C. on the 32nd day). These details are shown in Table V.

Figure 6.

Buffalo Ferdinand
"T. lawrencei (Kenya)" infection

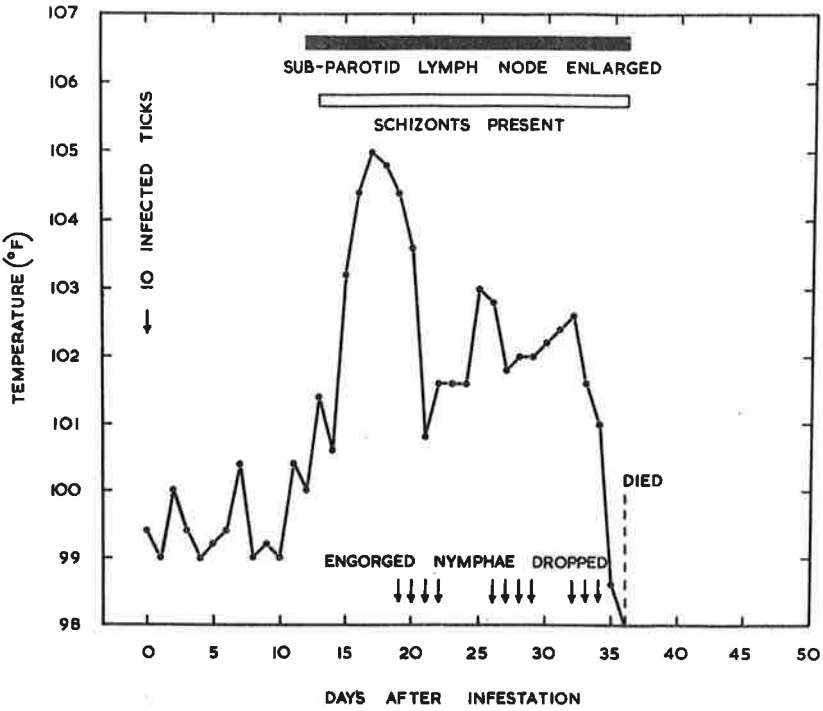

SUB-PAROTID LYMPH NODE ENLARGED
SCHIZONTS PRESENT
10 INFECTED TICKS
DIED
ENGORGED NYMPHAE DROPPED
TEMPERATURE (°F)
DAYS AFTER INFESTATION
107
106
105
104
103
102
101
100
99
98
0
5
10
15
20
25
30
35
40
45
50

Table V

Parasitosis and parasitaemia in Buffalo Ferdinand (fatal "T. lawrencei (Kenya)" infection)

Day	Sub-parotid node (S/1000L)	Prescapular node (S/1000L)	Blood (piros./1000 R.B.C.)
13	20	-	N.P.S.
14	116	-	N.P.S.
15	95	-	N.P.S.
16	214	18	N.P.S.
17	133	-	N.P.S.
18	40	44	N.P.S.
19	85	16	N.P.S.
20	150	84	N.P.S.
21	125	8	<1
22	293	9	<1
23	402	28	<1
24	439	11	<1
25	455	16	1
26	386	17	8
27	338	-	16
28	340	6	18
29	204	8	16
30	272	526	22
31	-	5	18
32	-	-	30
33	-	-	4
34	-	-	8
35	-	24	6
36	-	115	4

S/1000L = Schizonts per thousand lymphocytes.

The extraordinarily high count in the prescapular node on the 30th day is difficult to explain; perhaps the biopsy needle struck the centre of an unusually intense focus of schizogony.

The extensive multiplication of the parasite in this buffalo provided an opportunity for a more thorough study of the schizonts. The results concerning the number of nuclei within schizonts are shown in Table VI.

The average number of nuclei per schizont in biopsy smears was 6.47. This compares with figures of 9.27 and 7.14 n/s for T. parva (South African strain) and T. parva (Muguga) respectively. In autopsy smears the average figure was 9.55 compared with 10.82 and 7.79 for the two strains of T. parva. The total average (of 1700 schizonts) was 7.08 n/s., with a range of 1-90, compared with a figure of 7.97 for the two strains of T. parva. This tendency for the schizonts in the tissues of the buffalo to be smaller than those of T. parva is emphasised if we consider the frequency with which schizonts with 5 or fewer nuclei occur. Thus in all smears of the buffalo 55% of schizonts had 5 nuclei or less; the figure for T. parva was 36%.

One hundred schizonts were measured and found to average 3.98μ, varying between 2.0μ and 7.2μ in size.

Apart from this tendency for the schizonts to be smaller than those of T. parva, there were no differences.

Table VI

The number of nuclei within schizonts of "T. lawrencei (Kenya)" in Buffalo Ferdinand

No. of nuclei	Biopsy smears (1400 schizonts)		Autopsy smears (300 schizonts)		Total: All smears (1700 schizonts)	
	Actual Fig.	%	Actual Fig.	%	Actual Fig.	%
1	13	0.9	2	0.7	15	0.9 (0.4)
2	130	9.3	19	6.3	149	8.8 (3.4)
3	217	15.5	34	11.3	251	14.8 (8.0)
4	247	17.6	44	14.7	291	17.1(12.3)
5	184	13.1	38	12.7	222	13.1(11.9)
6	165	11.8	19	6.3	184	10.8(11.2)
7	103	7.4	11	3.7	114	6.7 (9.3)
8	84	6.0	21	7.0	105	6.2 (7.4)
9	36	2.6	15	5.0	51	3.0 (6.0)
10	39	2.8	11	3.7	50	2.9 (5.1)
11	26	1.9	14	4.7	40	2.4 (4.2)
12	27	1.9	8	2.7	35	2.1 (3.9)
13	19	1.4	7	2.3	26	1.5 (2.8)
14	21	1.5	10	3.7	31	1.8 (2.3)
15	8	0.6	6	2.0	14	0.8 (1.8)
16	18	1.3	5	1.7	23	1.4 (1.5)
17	8	0.6	5	1.7	13	0.8 (1.2)
18	13	0.9	3	1.0	16	0.9 (1.0)
19	3	0.2	2	0.7	15	0.9 (0.9)
20	9	0.6	4	1.3	13	0.8 (0.9)
21	1	0.1	3	1.0	4	0.2 (0.6)
22	1	0.1	1	0.3	2	0.1 (0.6)
23	5	0.4	1	0.3	6	0.4 (0.4)
24	1	0.1	1	0.3	2	0.1 (0.4)
25	1	0.1	2	0.7	3	0.2 (0.3)
26+	21	1.2	14	4.7	35	2.1 (2.2)
Average	6.47 n/s		9.55 n/s		7.08 n/s (7.97)	
Range	1 - 75		1 - 90		1 - 90 (1-85)	

The bracketed figures in the last column are the figures for

Buffalo Brutus

This animal had one year previously recovered from infection with T. parva (see above) and subsequently challenged with the same organism and found to be solidly immune. It had also been established that the buffalo had not become a carrier of T. parva.

Ten of the adult ticks that had dropped from Buffalo Nero on day 31 (Fig. 5) were allowed to feed on Buffalo Brutus. After an incubation period of 12 days the buffalo underwent a mild febrile reaction and recovered (Fig. 7). The superficial lymph nodes became enlarged and hypertrophic and macroschizonts were demonstrable on days 13, 14, 15, 19 and 20. They were always rare though more frequent than in the animal's earlier infection with T. parva. Neither microschizonts nor intra-erythrocytic piroplasms were produced. Uninfected nymphae were placed on the buffalo but unfortunately they failed to feed. It was therefore not established whether the buffalo was infective to ticks at the time of its febrile reaction. It was subsequently demonstrated that Buffalo Brutus did not become a carrier of "T. lawrencei (Kenya)". Engorged nymphae which dropped on days 60 and 62 failed to transmit the parasite to cattle; they were used as follows:-

20 ticks on to Steer No. 3332. No reaction resulted. The steer was later challenged with T. parva and found to be immune.

20 ticks on to Steer No. 4474. No reaction resulted. The steer was later challenged with T. parva and died.

50 ticks on to Steer No. 4463. No reaction resulted. The steer was later challenged with T. parva and died.

This case showed that the immunity conferred by previous infection and challenge with T. parva did not completely protect the buffalo from infection with "T. lawrencei (Kenya)".

Figure 7

Buffalo Brutus. "*T. lawrencei* (Kenya)"
infection one year after recovery from *T. parva*

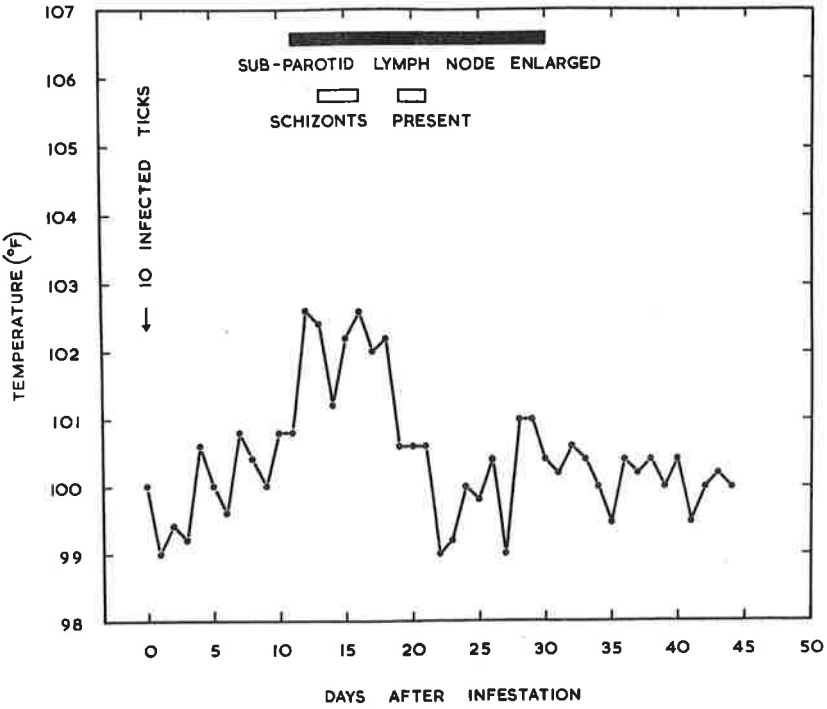
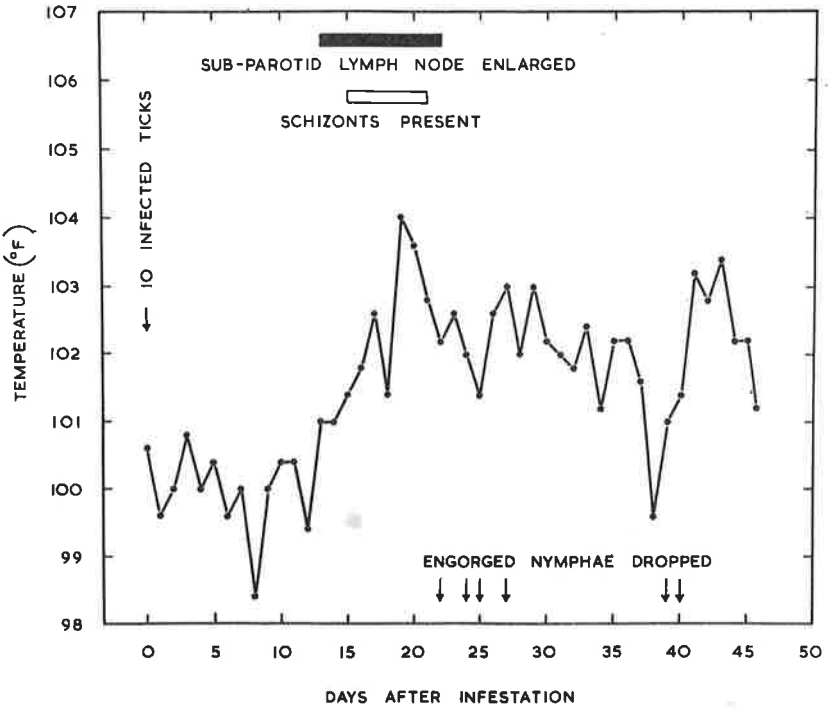

SUB-PAROTID LYMPH NODE ENLARGED
SCHIZONTS PRESENT
← 10 INFECTED TICKS
TEMPERATURE (°F)
107
106
105
104
103
102
101
100
99
98
0
5
10
15
20
25
30
35
40
45
50
DAYS AFTER INFESTATION

Figure 8

Buffalo Karim.
"*T. lawrencei* (Kenya)" infection.


SUB-PAROTID LYMPH NODE ENLARGED
SCHIZONTS PRESENT
10 INFECTED TICKS
TEMPERATURE (°F)
107
106
105
104
103
102
101
100
99
98
ENGORGED NYMPHAE DROPPED
0
5
10
15
20
25
30
35
40
45
50
DAYS AFTER INFESTATION

Buffalo Karim

This animal was a young male calf obtained from the Queen Elizabeth Park, Uganda.

Rare theilerial piroplasms were present in the blood of this buffalo. Twenty-five ticks failed to transmit this organism to Steer No. 4205 which unfortunately was not later challenged with T. parva. It could therefore only be tentatively concluded that the buffalo was not a carrier of T. parva or T. lawrencei.

Ten of the adult ticks that had dropped as engorged nymphae from Buffalo Ferdinand on the 28th day of his infection were allowed to feed on Buffalo Karim. After an incubation period of 17 days the buffalo underwent a mild febrile reaction lasting for about 6 days and recovered (Fig. 8). The superficial lymph nodes became enlarged and hypertrophic and very rare macroschizonts were detectable in smears for six days. Microschizonts could not be found and there was little, if any, production of piroplasms. Uninfected nymphae were placed on the buffalo and dropped engorged on the days shown in Fig. 8. Unfortunately these ticks could not be persuaded to feed on a fifth buffalo, Brenda, so with Karim the buffalo passage of the parasite came to an end. (The ticks did, however, transmit the parasite to cattle).

It was not established whether Buffalo Karim became a carrier of T. lawrencei (Kenya) as many attempts to feed nymphae on him were all unsuccessful.

It appears that the African Buffalo is more susceptible to infection with T. lawrencei than it is to infection with cattle strains of T. parva. This is emphasised if Table III is compared with Table VII, which summarises the available information on the susceptibility of the African buffalo to infection with T. lawrencei.

Table VII

The Susceptibility of the Buffalo to T. lawrencei

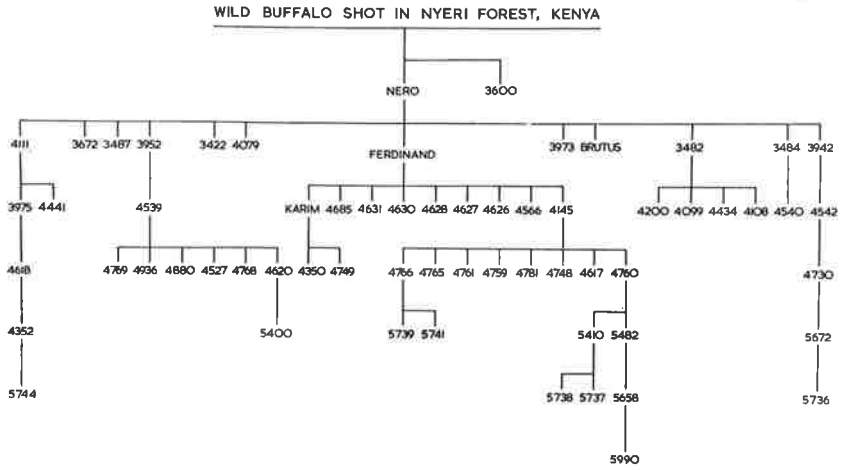
Ref.	Number of Buffaloes	Methods of infection	Result
Neitz (1957)	3	(i))Found infected (ii)) in nature	Both died.
		(iii) Ticks on ear	Slight reaction; (fever?); L/N enlarged; rare schizonts.
This paper	4	(i) Ticks on ear	Severe fever; L/N enlarged; frequent schizonts; transmissible to cattle. Died.
		(ii) Ticks on ear	Slight fever; L/N enlarged; rare schizonts; transmissible to cattle; carrier status unchecked.
		(iii) Ticks on ear	Slight fever; L/N enlarged; rare schizonts; did not become a carrier.
		(iv) Ticks on ear	Fairly severe fever; L/N enlarged; rare schizonts; transmissible to cattle; did not become a carrier.

L/N = Superficial lymph nodes.

From Table VII it can be seen that out of 7 buffaloes infected with the disease 3 (43%) have died; the other 4 had fairly mild or very mild infections in which schizonts were demonstrable. Three of the four infections described in this paper were transmitted to cattle.

Figure 9.

The Passage of "*T. lawrencei* (Kenya)"
through cattle and buffaloes


WILD BUFFALO SHOT IN NYERI FOREST, KENYA
NERO
3600
4111
3672 3487 3952
3422 4079
FERDINAND
3973 BRUTUS
3482
3484 3942
3975 4441
4539
KARIM 4685 4631 4630 4628 4627 4626 4566 4145
4200 4099 4434 4108 4540 4542
4618
4769 4936 4880 4527 4768 4620 4350 4749
4766 4765 4761 4759 4781 4748 4617 4760
4730
4352
5400
5739 5741
5410 5482
5672
5744
5738 5737 5658
5736
5990

THE PASSAGE OF "THEILERIA LAWRENCEI (KENYA)" THROUGH CATTLE

All transmissions were carried out using the tick R. appendiculatus as the vector; ticks were infected as nymphae and transmitted the disease by feeding when adults. R. appendiculatus appeared to be an efficient vector which was to be expected since it was in this species that the parasite was first isolated. It can be seen from Fig. 9 that 59 grade cattle were successfully infected: many more cattle were used in these experiments but for various reasons failed to become infected with "T. lawrencei (Kenya)". Only definite cases, proved to be infected by the discovery of schizonts in tissue smears, are discussed.

It is obviously impossible to describe each case in detail. Tables VIII - XII give in summary form an outline of each of the 59 cases. Particular cases will be discussed in more detail later but for the moment the general character of the disease as seen in the 59 cases will be described. Clinically the disease did not significantly differ from East Coast fever.

Figure 9 shows all the positive transmissions made with "T. lawrencei (Kenya)".

Table VIII

Summary of Cases in 1st and 2nd Passages in Cattle

	1st	2nd Passage				
Animal No.	3600	3942	3482	3484	3672	3973
No. of ticks	10	1	20	10	1	5
Incubation (days)	14	12	14	13	10	11
Reaction (days)	7	11	15	10	8	9
Max. Temp.	106.2	104.8	105.4	104.6	104.2	105.6
Result	D	D	R	R	R	D
Result of Challenge	-	-	-	I	-	-
Nuclei/ Schizont	6.73 (500)	5.47 (250)	6.88 (700)	6.73 (180)	3.18 (30)	6.70 (200)
Size of Schizonts (μ)	3.96 (500)	3.06 (100)	4.42 (100)	4.30 (100)	2.79 (30)	3.33 (100)
Max. piro. count	NPS	NPS	14	4	NPS	NPS
Max. macro-schizont count	20	12	74	4	4	5
Max. micro-schizont count	0	1	0	<1	0	<1

/contd.....

Table VIII contd.

2nd Passage contd.

Animal No.	3952	4079	3487	3422	4111
No. of ticks	3	10	10	10	10
Incubation (days)	17	19	11	-	11
Reaction (days)	16	7	5	-	10
Max. Temp.	104.2	106.2	106.6	-	106.2
Result	R	D	D	R	R
Result of Challenge	I	-	-	R	R
Nuclei/ Schizont	-	8.66 (350)	4.79 (550)	-	4.62 (400)
Size of Schizonts (μ)	-	4.04 (100)	2.95 (100)	-	4.05 (100)
Max. piro. count	NPS	NPS	NPS	<1	<1
Max. macro-schizont count	<1	92	95	<1	30
Max. micro-schizont count	0	<1	0	0	0

Bracketed figures = the number of schizonts examined or measured.

D = Died. R = Reacted and Recovered. I = Solidly immune.

NPS = No Parasites Seen.

Table IX

Summary of Cases in the 3rd Passage in Cattle

	3rd Passage					
Animal No.	4200	4099	4108	4434	4540	4539
No. of ticks	90	60	20	50	100	50
Incubation (days)	17	21	19	-	15	14
Reaction (days)	3	1	4	-	14	20
Max. Temp.	104.2	103.4	103.6	-	104.8	105.8
Result	R	R	R	R	R	D
Result of Challenge	I	I	D	D	I	-
Nuclei/ Schizont	-	-	7.02 (300)	-	6.64 (400)	3.57 (280)
Size of Schizonts (μ)	-	-	5.07 (100)	-	4.67 (100)	3.64 (100)
Max. piro. count	<1	<1	<1	<1	4	2
Max. macro-schizont count	<1	<1	24	<1	24	48
Max. micro-schizont count	0	0	4	0	1	0

/contd.....

Table IX contd.

3rd Passage

Animal No.	4542	3975	4441	4145	4566	4626
No. of ticks	150	45	10	40	1	1
Incubation (days)	14	15	14	12	20	14
Reaction (days)	11	17	7	9	7	6
Max. Temp.	105.0	105.4	106.4	105.2	107.0	105.4
Result	D	D	D	D	D	R
Result of Challenge	-	-	-	-	-	I
Nuclei/ Schizont	8.69 (300)	5.38 (100)	4.46 (250)	4.29 (750)	6.19 (200)	-
Size of Schizonts (μ)	4.65 (100)	4.17 (100)	2.63 (100)	4.05 (100)	3.63 (100)	-
Max. piro. count	72	<1	NPS	<1	<1	NPS
Max. macro-schizont count	1410	4	920	119	96	<1
Max. micro-schizont count	68	0	0	0	1	0

/contd.....

Table IX contd.

3rd Passage

Animal No.	4627	4628	4630	4631	4688
No. of ticks	1	3	5	10	10
Incubation (days)	13	23	7	14	10
Reaction (days)	13	7	13	8	12
Max. Temp.	106.0	104.8	105.4	105.8	106.2
Result	R	R	D	R	D
Result of Challenge	R	R	-	I	-
Nuclei/ Schizont	-	4.16 (50)	-*	5.19 (100)	3.79 (100)
Size of Schizonts (μ)	-	3.01 (50)	-*	3.45 (100)	3.37 (100)
Max. piro. count	<1	<1	-*	25	NPS
Max. macro-schizont count	3	5	-*	36	24
Max. micro-schizont count	0	0	-*	0	0

* Slides lost

Table X

Summary of Cases in the 4th Passage in Cattle

	4th Passage					
Animal No.	4620	4527	4936	4680	4769	4768
No. of ticks	50	20	20	10	10	20
Incubation (days)	21	18	16	20	13	17
Reaction (days)	3	5	6	7	16	5
Max. Temp.	104.2	105.2	104.2	105.6	105.8	105.6
Result	R	R	R	D	D	D
Result of Challenge	I	R	-	-	-	-
Nuclei/ Schizont	-	-	-	5.01 (100)	4.98 (100)	4.11 (1200)
Size of Schizonts (μ)	-	-	-	4.05 (100)	4.02 (80)	3.76 (100)
Max. piro. count	<1	3	<1	NPS	<1	<1
Max. macro-schizont count	<1	<1	<1	40	4	86
Max. micro-schizont count	0	0	0	0	0	0

/contd.....

Table X contd.

4th Passage

Animal No.	4730	4618	4748	4781	4617	4759
No. of ticks	20	50	20	10	10	20
Incubation (days)	13	11	9	17	-	15
Reaction (days)	13	10	8	1	-	8
Max. Temp.	106.6	107.2	104.4	105.0	-	105.6
Result	D	D	R	D	R	R
Result of Challenge	-	-	R	-	I	-
Nuclei/ Schizont	11.6 (200)	6.79 (450)	-	6.97 (200)	-	8.29 (100)
Size of Schizonts (μ)	6.07 (100)	5.80 (100)	-	4.60 (110)	-	3.82 (100)
Max. piro. count	1104	42	4	<1	<1	21
Max. macro-schizont count	948	630	<1	56	<1	26
Max. micro-schizont count	28	20	0	0	0	4

/contd.....

Table X contd.

4th Passage

Animal No.	4760	4761	4765	4766	4749	4350
No. of ticks	20	20	20	20	10	10
Incubation (days)	14	14	12	17	14	14
Reaction (days)	14	3	9	2	6	8
Max. Temp.	106.8	104.8	103.4	102.2	102.8	107.2
Result	R	R	R	R	R	D
Result of Challenge	I	-	I	-	-	-
Nuclei/ Schizont	6.45 (400)	-	5.74 (100)	-	-	4.64 (200)
Size of Schizonts (μ)	4.66 (100)	-	3.59 (100)	-	-	3.89 (100)
Max. piro. count	68	33	12	4	<1	<1
Max. macro-schizont count	81	<1	10	<1	<1	196
Max. micro-schizont count	14	0	<1	0	0	<1

Table XI

Summary of Cases of the 5th Passage

	5th Passage						
Animal No.	5400	5672	4352	5410	5482	5739	5741
No. of ticks	10	20	10	20	10	30	30
Incubation (days)	11	7	13	17	19	14	13
Reaction (days)	6	8	12	12	6	20	8
Max. Temp.	106.0	105.0	106.8	103.2	104.4	104.6	105.8
Result	D	R	D	R	R	D	R
Result of Challenge	-	I	-	-	I	-	I
Nuclei/ Schizont	5.22 (280)	-	10.50 (400)	7.05 (125)	8.69 (400)	7.08 (200)	5.32 (200)
Size of Schizonts (μ)	4.17 (100)	-	5.68 (100)	4.50 (125)	4.06 (105)	4.57 (100)	4.17 (100)
Max. piro. count	8	50	618	33	78	79	2
Max. macro-schizont count	46	<1	594	38	26	56	18
Max. micro-schizont count	0	0	60	1	6	1	<1

Table XII

Summary of Cases in the 6th & 7th Passages in Cattle

	6th Passage					7th
Animal No.	5736	5744	5737	5738	5658	5990
No. of ticks	20	50	20	20	20	20
Incubation (days)	15	15	16	14	14	15
Reaction (days)	10	13	9	6	14	5
Max. Temp.	106.0	106.8	105.2	105.0	105.6	106.0
Result	D	D	R	R	R	R
Result of Challenge	-	-	R	I	-	I
Nuclei/ Schizont	7.66 (300)	9.31 (280)	6.45 (400)	5.45 (93)	6.60 (400)	7.73 (400)
Size of Schizonts (μ)	5.07 (100)	5.37 (100)	4.95 (100)	4.19 (93)	5.54 (100)	5.61 (100)
Max. piro. count	680	640	102	<1	132	28
Max. macro-schizont count	753	495	102	<1	131	254
Max. micro-schizont count	96	45	17	0	27	8

Morbidity Rate

Seventy cattle were infested with ticks of batches known to be infected and 59 became demonstrably infected with the parasite. The morbidity rate was therefore 84.3%. This compares with a figure of 87.6% for T. parva infections in similar cattle (Brocklesby et al., 1961).

Mortality Rate

Of the 59 cattle that became infected with the organism 25 died and 34 recovered. The mortality rate was therefore 42% which is much lower than the comparable figure for T. parva (95.5%). The mortality rate was not related to the number of ticks used to transmit the disease. [Such a relationship was demonstrated by Barnett and Brocklesby (1961) in the case of a mild strain of T. parva]. Table XIII demonstrates this.

Incubation Period

The incubation period was defined as the period from the day that ticks were placed on the animal to the day that the temperature first rose sharply, inclusive of both days. In most cases the temperature rose sharply to more than 103°F. but sometimes, more particularly in cattle with low normal temperatures, the first sharp rise was to less than 103°F. In rare instances the temperature rose very gradually and in these cases the incubation period was taken as ending on the day that the temperature rose to 103°F. or more.

Three of the cattle (Nos. 3422, 4434 and 4617) had no thermal reaction so that their Incubation and Reaction Periods could not be assessed. Table XIV shows the Incubation Periods of the 56 cases.

Table XIII

Relationship between Tick Numbers and Mortality

Number of ticks used	Number of cattle that died	Number of cattle that recovered	Mortality Rate
1	2	3	
3		2	44%
5	2		
10	11	7	
20	3	16	37%
30	1	1	
40	1		
45	1		
50	3	2	
60		1	54%
90		1	
100		1	
150	1		

Table XIV

Incubation Period in Cattle

Incubation period (days)	Number of cases	% of cases
7	2	3.6 (-)
8	-	- (-)
9	1	1.8 (-)
10	2	3.6 (2.7)
11	5	8.9 (6.0)
12	3	5.4 (16.7)
13	6	10.7 (22.7)
14	14	25.0 (26.0)
15	6	10.7 (12.0)
16	2	3.6 (5.3)
17	6	10.7 (4.0)
18	2	3.6 (1.3)
19	2	3.6 (1.3)
20	2	3.6 (-)
21	2	3.6 (-)
22	-	- (-)
23	1	1.0 (1.3)
24	-	- (-)
25	-	- (0.7)

The bracketed figures are those for fatal *T. parva* infections in similar cattle all infected with 10 ticks (Brocklesby, 1962).

The Incubation Period varied from 7-23 days with an average of 14.5 days. From Table XIV it can be seen that in 89% of cases the incubation period was 9 - 19 days, in 75% of cases the incubation period was 11 - 17 days and in 44% of cases the incubation period was 13 - 15 days.

For the 25 fatal cases the incubation period averaged 13.8 days and for the 31 cases that recovered it averaged 15 days.

There was no correlation between the number of ticks used and the length of the Incubation Period.

Reaction Period

In the case of fatal cases the Reaction Period was defined as the period from the first day of fever (i.e. the last day of the Incubation Period) to the day of death inclusive. Most cattle died during the night and their "day of death" was taken to be the day on which they were found dead. For the cattle that recovered the Reaction Period was defined as the period from the first day of fever until the last day that the temperature was elevated (usually to more than 103°F.). Table XV shows the Reaction Periods of the 56 cases.

The Reaction Period in fatal cases varied from 1 - 20 days with an average of 10 days. In cattle which recovered it varied from 1 - 16 days with an average of 8 days.

From Table XV it can be seen that:

in 85% of cases the reaction period was 3 - 14 days,
in 67% of cases the reaction period was 3 - 11 days, and
in 49% of cases the reaction period was 5 - 9 days.

Table XV

Reaction Period in Cattle

Reaction Period (days)	Number of cases		% of cases
	Fatal	Recovered	
1	1	1	3.6 (-)
2		1	1.8 (-)
3		3	5.4 (-)
4		1	1.8 (0.7)
5	2	2	7.2 (0.7)
6	1	5	10.8 (1.3)
7	5	1	10.8 (2.7)
8	1	6	12.6 (5.3)
9	2	2	7.2 (6.0)
10	2	2	7.2 (8.0)
11	2		3.6 (9.3)
12	2	1	5.4 (20.0)
13	3	1	7.2 (15.3)
14		3	5.4 (8.0)
15		1	1.8 (10.7)
16	1	1	3.6 (3.3)
17	1		1.8 (4.0)
18			- (3.3)
19			- (1.3)
20	2		3.6 (-)

The bracketed figures are those for fatal *T. parva* infections (Brocklesby, 1961).

Remissions of Fever

This character was defined as the number of days during the Reaction Period that the temperature fell to a certain level. Two levels were used: (a) the number of days that the temperature fell below 103°F. and (b) the number of days that the temperature fell below 102°F. Table XVI shows the remissions of fever that occurred in the 56 cases.

From Table XVI it can be seen that 14 cases (25%) had remissions of fever (below 103°F.) during the reaction period. Five of these were double remissions and one was a triple remission (with the animal being afebrile for 6 days before death). In only 7 cases (12.5%) did the temperature fall to below 102°F.

/Table XVI...

Table XVI

Remissions of Fever in Cattle

Animal number	Days of Remission during Reaction	
	Below 103°F.	Below 102°F.
3482	5 + 1	3
3484	1	-
3672	1	-
3952	4	4
4200	1	1
4108	1	1
4539	4 + 6	6
3975	4 + 1 + 6	6
4626	1	1
4627	1 + 1	-
4630	1	-
4688	1 + 1	-
4680	1	-
5739	3 + 1	-

Variety of Febrile Reactions

From the above remarks and from Tables VIII - XII it is evident that the parasite caused a wide variety of thermal responses in the infected cattle. As has already been mentioned three of the cattle had no febrile reaction at all; parasites could nevertheless be demonstrated in smears made from the slightly enlarged superficial lymph nodes.

Four of the thermal reactions encountered are shown in Figure 10 which illustrates the wide variety of febrile reactions.

Cross Immunity with Theileria parva

Since Neitz (1957) reported that a good cross immunity existed between the South African strains of T. parva and T. lawrencei, it was decided to see whether the same was true for the Kenya strains. Twenty-five of the cattle which recovered from infection with "T. lawrencei (Kenya)" were challenged with our laboratory strain of T. parva. Some were challenged immediately after recovery and others 2, 2½ and 3 years after recovery.

Eleven of the cattle were challenged immediately and eight of them were solidly immune; one of them reacted and recovered and the other two reacted and died. These two cattle (Nos. 4108 and 4434) had undergone extremely mild infections with "T. lawrencei (Kenya)"; similar reactions in Nos. 3422 and 4617 did, however, apparently immunize them.

Eight cattle were challenged 2 years after recovery. Six of these were solidly immune and two reacted and recovered. Two cattle were challenged 2½ years after recovery and both reacted and recovered. Four cattle were challenged 3 years after recovery; two were solidly immune and two reacted and recovered.

It can therefore be concluded that infection with "T. lawrencei (Kenya)" did provide a strong immunity to subsequent infection with T. parva and that this immunity lasted for at least 3 years.

Parasitaemia

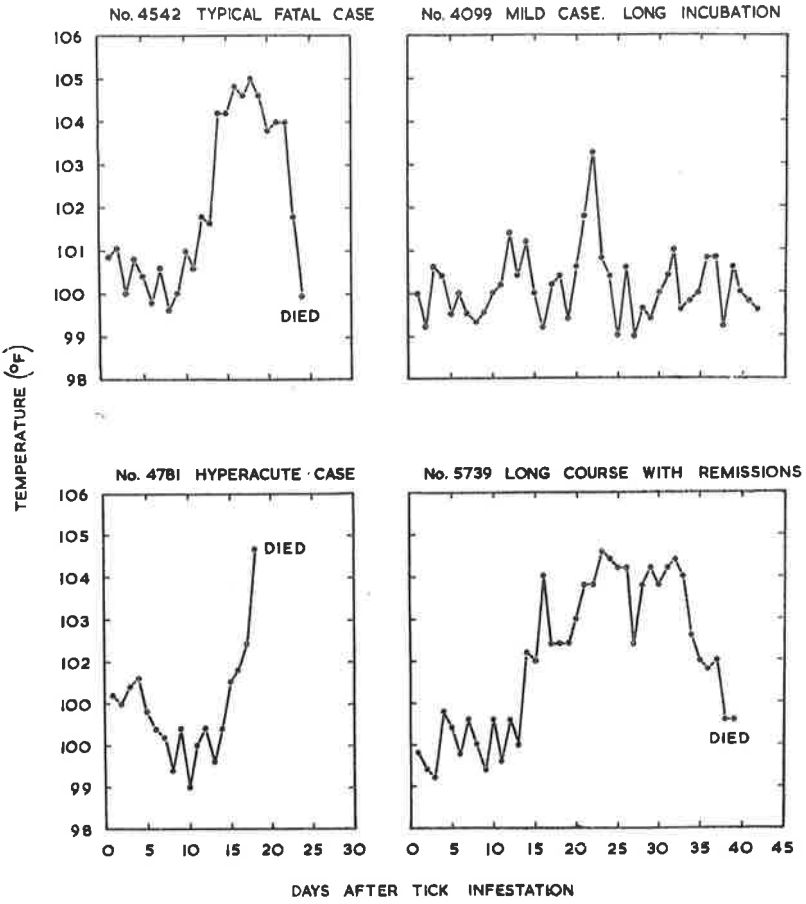
Thin blood films were examined from all experimental cattle each day during the reaction period. Where theilerial piroplasms were present they were counted, the result being expressed as piroplasms per thousand red blood cells. It must be mentioned here that most cattle in East Africa are carriers of the benign T. mutans and piroplasms of this species are frequently seen in thin blood films prepared from both healthy and sick cattle. When present they are usually extremely rare (<1/1000 R.B.C.) so that they do not interfere with studies on the pathogenic species which, in this context, are concerned with whether or not piroplasms were produced in a particular case.

It will be recalled that one of the characters of T. lawrencei in South Africa and Rhodesia was the fact that piroplasms were either not produced or were very rare. It was therefore important to know whether or not this applied to "T. lawrencei (Kenya)".

From Table VIII it can be seen that in 7 out of the 11 cattle of the 1st and 2nd passages no piroplasms were seen; in 3 others they were extremely rare (probably T. mutans); in only one animal (No. 3482) was there any significant production of piroplasms (14/1000 R.B.C.) and this was very much less than is usually seen in East Coast fever. The parasite, from the point of view of piroplasm production, in cattle of the 1st and 2nd passages therefore behaved like T. lawrencei. However, in spite of the fact that careful and prolonged examination of thin blood films failed to reveal any piroplasms, it was still possible to transmit the organism with ticks (Nos. 3949 and 3952, see Fig.9).

Figure 10

Some of the Febrile Reactions seen in Cattle infected with "*T. lawrencei* (Kenya)"


No. 4542 TYPICAL FATAL CASE
No. 4099 MILD CASE. LONG INCUBATION
No. 4781 HYPERACUTE CASE
No. 5739 LONG COURSE WITH REMISSIONS
DIED
DIED
DIED
TEMPERATURE (°F)
106
105
104
103
102
101
100
99
98
0 5 10 15 20 25 30
0 5 10 15 20 25 30 35 40 45
DAYS AFTER TICK INFESTATION

When the 17 cases of the 3rd passage are examined (Table IX) it is seen that no piroplasms could be detected in 3 cattle and very low counts occurred in a further 12 animals. However, in 2 of the cattle (Nos. 4542 and 4631) piroplasm production did take place (up to 25 and 72/1000 R.B.C.). Once again it was possible to transmit the parasite with ticks from animals with very low piroplasm counts (Nos. 3975 and 4145, see Fig. 9).

The tendency for piroplasm production to increase with the passage of the organism through cattle became marked in the 18 cattle of the 4th passage (Table X). In only one animal were no piroplasms seen and very low counts occurred in a further 11 cattle. In six of the animals, piroplasm production took place and in one of these (No. 4730) it was copious (up to 1104/1000 R.B.C.). It was again possible to transmit the organism with ticks that had engorged on an animal with a very low piroplasm count (No. 4620, see Fig. 9).

In the 13 cattle of the 5th, 6th and 7th passages, piroplasm production had become the rule rather than the exception (Tables XI and XII). Low counts occurred in only 3 of the cattle and in the remainder counts were high (up to 680/1000 R.B.C.).

It appeared, therefore, that the ability of "T. lawrencei (Kenya)" to complete its life-cycle in cattle increased with its passage through them.

Parasitosis

Estimations of parasitosis were carried out on lymph node smears from all the infected cattle. These smears were prepared from sub-parotid and prescapular lymph nodes and were examined each day throughout the course of the disease.

One of the characters of the South African and Rhodesian strains of T. lawrencei that differentiated them from T. parva was the fact that few schizonts were produced; microschizonts were particularly rare. Tables VIII - XII show the maximum macroschizont and microschizont counts encountered in each case. They are expressed as schizonts per thousand lymphocytes.

In cattle of the 1st and 2nd passages (Table VIII) the schizont counts were low and well within the range given for the South African strain of T. lawrencei. However, with passage through cattle this character also changed (Tables IX, X and XI) until, by the 6th passage (Table XII), both macroschizonts and microschizonts were frequent (with one exception, No. 5738).

The Morphology of Macroschizonts

The extensive observations on the macroschizonts were carried out in the belief that the parasite was T. lawrencei. It was hoped that these observations could be compared statistically with those reported by Barnett et al. (1961) for T. parva and that differential diagnostic criteria could be elaborated. Indeed much effort was spent in the design of a scheme for differential diagnosis by the sequential sampling of schizonts based on the numbers of nuclei that they contained.

When it became obvious that "T. lawrencei (Kenya)" was changing its character on passage through cattle the observations were continued in order to follow the changes in detail. There would now appear to be no purpose in comparing the figures obtained for "T. lawrencei (Kenya)" with those obtained for T. parva as the former has apparently become changed into the latter.

(a) The Size of Macroschizonts (Tables VIII - XII)

A total of 4,493 macroschizonts was measured. Generally the lymph node smears used were those taken on the day of maximum parasitosis and an effort was made to measure at least 100 macroschizonts from each case. It was impossible to measure a reasonable number of schizonts from some cases since parasites were so rare. If the macroschizont count was 4/1000 lymphocytes or more it was usually possible to glean 100 parasites for measuring. The main objects of this laborious procedure were firstly to see whether "T. lawrencei (Kenya)" differed in this character from T. parva and, secondly, to see whether the changes already mentioned would be reflected in a change in size of the parasites.

The average size of the 4,493 schizonts was 4.25μ which is fairly close to the figure of 4.8μ given by Barnett et al. (1961) for 1750 macroschizonts of T. parva.

The average schizont size increased during the passage of the parasite through cattle:-

1st Passage:	Average of 500 parasites	=	3.96 μ
2nd Passage:	Average of 730 parasites	=	3.70 μ
3rd Passage:	Average of 1050 parasites	=	3.89 μ
4th Passage:	Average of 990 parasites	=	4.46 μ
5th Passage:	Average of 630 parasites	=	4.52 μ
6th Passage:	Average of 493 parasites	=	5.04 μ
7th Passage:	Average of 100 parasites	=	5.61 μ

i.e. the average size of the schizonts of "T. lawrencei (Kenya)" increased from 3.96μ to 5.61μ from the 1st to the 7th passage in cattle. This is an increase of 42%.

The average schizont size varied considerably from case to case (from 2.63μ to 6.07μ).

The smallest schizont measured 0.8μ by 0.8μ and the largest measured 17.6μ by 16.8μ.

(b) The Number of Nuclei within Macroschizonts (Tables VIII - XII)

A total of 12,493 macroschizonts was included. The average number of nuclei was found to be 6.3 which was fewer than the figure (8) given by Barnett et al. (1961) for 12,000 macroschizonts of T. parva.

There was a tendency for the average numbers of nuclei to increase with the passage of the parasite through cattle:-

1st Passage:	Average of 500 parasites	=	6.73 n/s
2nd Passage:	Average of 2660 parasites	=	6.14 n/s
3rd Passage:	Average of 2830 parasites	=	5.51 n/s
4th Passage:	Average of 3030 parasites	=	5.75 n/s
5th Passage:	Average of 1600 parasites	=	7.80 n/s
6th Passage:	Average of 1473 parasites	=	7.22 n/s
7th Passage:	Average of 400 parasites	=	7.73 n/s

The average number of nuclei per schizont varied considerably from case to case (3.18 n/s to 11.60 n/s).

A Consideration of Particular Passage Lines

The changes did not take place consistently in all passage lines. Four particular passage lines are considered here (see Fig. 9). The characters considered are parasitaemia (piroplasms/1000 R.B.C.), parasitosis (schizonts/1000 lymphocytes), size (μ) and the number of nuclei within macroschizonts (n/s).

The passage lines are:-

Passage Line 1.	3952	--->	4539	-->	4620	-->	5400.	
Passage Line 2.	3942	--->	4542	-->	4730	-->	5672	--> 5736.
Passage Line 3.	4145	--->	4760	-->	5482	-->	5658	--> 5990.
Passage Line 4.	4111	--->	3975	-->	4618	-->	4352	--> 5744.

The results are shown in Tables XVII, XVIII, XIX and XX.

Table XVII

Passage Line 1

Animal Number (Passage)	Maximum piroplasm count	Max.macro-schizont count	Max.micro-schizont count	Average size (μ)	Nuclei per schizont
3952 (2nd)	NPS	<1	0	-	-
4539 (3rd)	2	48	0	3.64	3.57
4620 (4th)	<1	<1	0	-	-
5400 (5th)	8	46	0	4.17	5.22

Table XVIII

Passage Line 2

Animal Number (Passage)	Maximum piroplasm count	Max.macro-schizont count	Max.micro-schizont count	Average size (μ)	Nuclei per schizont
3942 (2nd)	NPS	12	1	3.06	5.47
4542 (3rd)	72	1410	68	4.65	8.69
4730 (4th)	1104	948	28	6.07	11.6
5672 (5th)	50	<1	0	-	-
5736 (6th)	680	753	96	5.07	7.66

Table XIX

Passage Line 3

Animal Number (Passage)	Maximum piroplasm count	Max.macro-schizont count	Max.micro-schizont count	Average size (μ)	Nuclei per schizont
4145 (3rd)	<1	119	0	4.05	4.29
4760 (4th)	68	81	14	4.66	6.45
5482 (5th)	78	26	6	4.06	8.69
5658 (6th)	132	131	27	5.54	6.60
5990 (7th)	28	254	8	5.61	7.73

Table XX

Passage Line 4

Animal Number (Passage)	Maximum piroplasm count	Max.macro-schizont count	Max.micro-schizont count	Average size (μ)	Nuclei per schizont
4111 (2nd)	<1	30	0	4.05	4.62
3975 (3rd)	<1	4	0	4.17	5.38
4618 (4th)	42	630	20	5.80	6.79
4352 (5th)	195	594	60	5.68	10.50
5744 (6th)	640	495	45	5.37	9.31

Before these 4 passage lines are discussed, it is necessary to recapitulate the characters that Neitz (1957) considered to be diagnostic of T. lawrencei.

> "... a definite diagnosis depends upon demonstrating the schizonts of G. lawrencei in blood and organ smears. The Koch bodies of G. lawrencei vary from 1.0 to 10.0 microns with an average diameter of 5.0 microns ..... The Koch bodies of Th. parva and G. mutans vary from 1.0 to 15.0 microns with an average diameter of 8.0 microns ..... In Corridor disease.. approximately five per cent of lymphocytes are parasitized with Koch bodies but mature microschizonts (gamonts) liberating merozoites are encountered only on rare occasions .... in East Coast fever usually more than 60 per cent of lymphocytes harbour macro- and microschizonts".

Neitz has only seen erythrocytic parasites of T. lawrencei in buffaloes or in splenectomised cattle. It must also be recalled that Barnett et al. (1961) found that the average size of schizonts of T. parva was 4.8µ, which was actually smaller than the figure given by Neitz as the average for T. lawrencei.

If the differential diagnostic criteria propounded by Neitz are applied to the cases in Tables XVII - XX it is seen that all the cases of Passage line 1 (Table XVII) would be diagnosed as being due to T. lawrencei. There was, therefore, no change in this passage line; the piroplasms seen were almost certainly T. mutans.

Passage line 2 (Table XVIII) showed a sudden change from T. lawrencei to T. parva between the 2nd and 3rd Passages. The low figures in Case No. 5672 can be explained by the fact that the animal suffered only a very mild infection; the piroplasm count would, nevertheless, lead to a diagnosis of T. parva.

The cases in Passage line 3 (Table XIX) would cause difficulty in diagnosis. No. 4145 would almost certainly be diagnosed as being due to T. lawrencei and the others, because of the presence of microschizonts and intra-erythrocytic piroplasms, would be diagnosed as being due to T. parva.

The cases in Passage line 4 (Table XX) show the changes with great clarity. Cases 4111 and 3975 would certainly be diagnosed as being due to T. lawrencei and Cases 4618, 4352 and 5744 are typical East Coast fever reactions. The average size of the schizonts and the average number of nuclei per schizont also increased during the passage of the parasite.

The Carrier Status of Recovered Cattle

It was decided, since Neitz (1958a, 1958b) had demonstrated that the carrier state could be demonstrated in recovered cattle by splenectomy, to investigate the carrier status of cattle that recovered from infection with "T. lawrencei (Kenya)". All the work described here was carried out using intact cattle.

At varying intervals after recovery R. appendiculatus nymphae were allowed to engorge on the cattle. These ticks were then allowed to moult to adults when they were infested on fresh cattle. These recipient cattle were examined each day, according to the usual routine, for a period of six weeks. They were then challenged with T. parva in order to check their susceptibility. The results are shown in Table XXI.

Table XXI

Carrier Status of Cattle Recovered from Infection with "T. lawrencei (Kenya)"

Animal Number	Months after recovery that the test ticks dropped	Number of ticks used	Number of recipient animal	Result	Result of challenge with T.parva
4111	4	9	4350	N.R.	D.*
	4	20	4352	N.R.	D.
	4	20	4338	N.R.	D.
	11	7	5516	N.R.	R.
	17	34	5834	N.R.	D.
4628	1	12	4754	N.R.	D.
	15	55	6353	N.R.	D.
4627	1	26	4976	N.R.	I.
	2	27	4977	N.R.	D.
	15	65	6056	N.R.	I.
4540	2	27	4968	N.R.	D.
	2	39	4975	N.R.	D.
4766	3	47	5540	R.	R.
	8	23	6608	N.R.	D.
	11	24	6359	N.R.	D.
	15	23	6452	D.	-.
4527	2	33	5514	N.R.	D.
	8	15	5877	N.R.	D.
4748	1	20	5402	N.R.	D.
	4	9	5515	N.R.	D.
	10	19	5881	N.R.	D.
4749	1	19	5517	N.R.	D.
	4	58	5529	N.R.	D.
	10	51	5883	N.R.	I.
4761	3	52	5543	N.R.	D.
	7	31	5887	Sl.R.	R.
4936	4	47	5627	Sl.R.	-.
	8	8	5889	N.R.	D.

/contd.....

Table XXI contd.

Animal Number	Months after recovery that the test ticks dropped	Number of ticks used	Number of recipient animal	Result	Result of challenge with T.parva
4765		19	5888	N.R.	I.
5482	3	51	6617	N.R.	D.
4760	8	48	6607	N.R.	D.
4759	8	22	6590	Sl.R.	D.
5738	9	50	6862	N.R.	D.
5737	9	50	6903	N.R.	D.
4620	14	73	6010	N.R.	I.

N.R. = No Reaction. D. = Died.
R. = Reacted & Recovered. I. = Solidly Immune.
Sl.R. = Slight Reaction.

From Table XXI it can be seen that 5 out of 17 cattle were shown to have become carriers 3, 4, 7, 8 and 15 months after recovery. That this carrier state was not continuous can be seen from the case of No. 4766 which was demonstrated to be a carrier 3 and 15 months after recovery but not at 8 and 11 months.

Since this is the first time that intact cattle recovered from a tick-induced East Coast fever-like disease have been shown to become carriers of the causal parasite, the five cases produced by the carrier test ticks will be described in detail. One of these cases, No. 6590, was infective for ticks and resulted in further passage of the organism: this passage resulted in further extraordinary changes in the nature of the parasite and so will be described in some detail.

Carrier Case No. 5540.

After an incubation period of 18 days this steer underwent a fairly severe febrile reaction which lasted for 9 days. The maximum temperature was 105.2°F. The superficial lymph nodes were enlarged for 10 days and macroschizonts (up to 18/1000 lymphocytes) were present for this period; microschizonts were present (<1/1000 lymphocytes) for 5 days. Piroplasms were produced in small numbers (up to 9/1000 R.B.C.) and disappeared when the animal recovered. An attempt to transmit the parasite, *via* ticks, to another steer was unsuccessful. The animal was later challenged with *T. parva* and underwent a very mild reaction.

Carrier Case No. 6452.

An incubation period of 16 days was followed by a severe febrile reaction of 10 days duration, the maximum temperature being 106.0°F. The superficial lymph nodes were enlarged for 8 days. The steer became afebrile for 3 weeks but never recovered and was eventually killed *in extremis*. Macroschizonts (up to 142/1000 lymphocytes) were present in smears for 15 days and microschizonts (<1/1000 lymphocytes) were occasionally seen. Piroplasms were produced in quite large numbers (up to 162/1000 R.B.C.) but an attempt to transmit the parasite to two further steers, with ticks, was not successful.

Carrier Case No. 5627.

This animal showed no thermal response but 17 days after the test ticks were placed on it the sub-parotid lymph node became enlarged and remained so for 8 days. Very rare (<1/1000 lymphocytes) macroschizonts were found on only 1 day. There was no apparent production of microschizonts or piroplasms. The animal was later challenged with *T. parva* and died.

The parasite was transmitted, *via* ticks, to Steer No. 6135 which had, after an incubation period of 20 days, a mild

were present for 7 days in smears of the superficial lymph nodes, which were enlarged for 15 days. However, this infection did not immunize the steer against infection with T. parva for on immediate challenge it reacted and died.

Carrier Case No. 5887.

Twenty-three days after infestation this animal underwent a very mild febrile reaction (103.6°F.) which lasted for only 2 days. The superficial lymph nodes were enlarged for 5 days but macroschizonts were demonstrated on only 1 day (<1/1000 lymphocytes): microschizonts were not seen and there was no apparent production of piroplasms. An attempt to transmit the parasite to two steers was unsuccessful. The animal was later challenged with T. parva and reacted and recovered.

Carrier Case No. 6590.

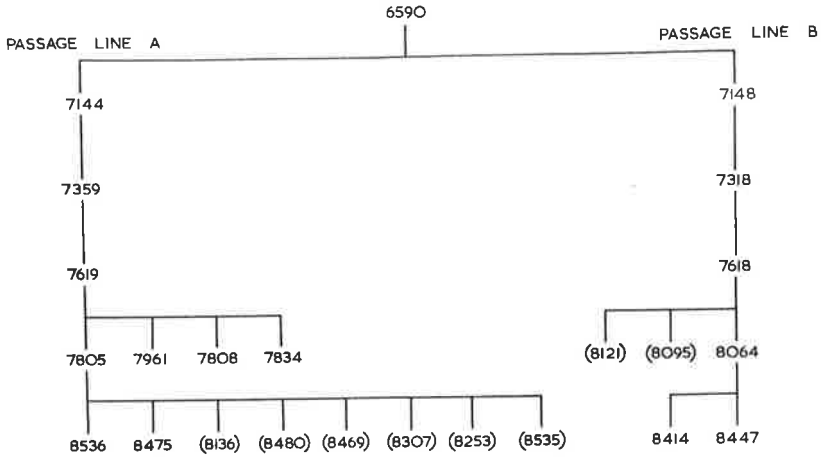
This case, with those that resulted from it, provided most interesting information concerning the lability of "T. lawrencei (Kenya)" and will, therefore, be described in some detail.

The disease produced in No. 6590 was very mild: no fever occurred but the sub-parotid lymph node was slightly enlarged for 3 days. Macroschizonts (up to 3/1000 lymphocytes) were present for 3 days: microschizonts were not detected. Piroplasms were not produced.

Uninfected nymphae of R. appendiculatus were allowed to feed on No. 6590 during the mild disease and, after moulting to adults, they were allowed to feed on two cattle, Nos. 7148 and 7144. Both these animals became infected and the parasite was transmitted through further cattle, via ticks as shown in Figure 11 and Tables XXII and XXIII. The passage line on the left in Figure 11, beginning with Steer No. 7144, will be referred to as "Passage line A" and the passage line on the right, beginning with Steer No. 7148, will be known as "Passage line B".

Figure 11

The Passage of "T. lawrencei (Kenya)"
from Carrier Case No.6590



THE BRACKETED ANIMALS DID NOT REACT

Passage line A

The results are summarised in Table XXII.

Table XXII

Summary of Cases in Passage Line A (see Fig. 11)

	1st	2nd	3rd
Animal No.	7144	7359	7619
No. of ticks	50	c.20	c.5
Incubation (days)	15	-	21
Reaction (days)	2-3	-	13
Max. Temp.	103.8	-	106.2
Result	R	R	D
Result of Challenge	D	I	-
Nuclei/ Schizont	5.26 (100)	12.26 (65)	10.05 (100)
Size of Schizonts (μ)	4.48 (100)	6.01 (65)	5.45 (100)
Max. piro. count	2	<1	181
Max.macro-schizont count	6	8	35
Max.micro-schizont count	0	0	0

/contd.....

Table XXII contd.

	4th				5th	
Animal No.	7961	7805	7808	7834	8475	8536
No. of Ticks	20	10	20	50	10	10
Incubation (days)	13	14	14	11	16	15
Reaction (days)	5	13	7	11	11	12
Max. Temp.	106.8	106.6	106.8	106.4	106.6	106.2
Result	D	D	D	D	R	D
Result of Challenge	-	-	-	-	I	-
Nuclei/ Schizont	8.71 (100)	9.87 (100)	11.29 (100)	8.59 (100)	9.89 (100)	8.26 (100)
Size of Schizonts (μ)	5.02 (100)	6.06 (100)	6.48 (100)	5.47 (100)	5.95 (100)	5.26 (100)
Max. piro. count	<1	486	194	59	167	167
Max.macro-schizont count	660	166	510	460	485	190
Max.micro-schizont count	10	0	30	12	15	10

Bracketed figures = the number of schizonts examined or measured

D = Died

R = Reacted and Recovered

I = Solidly Immune

Steers Nos. 6590 and 7144 underwent very mild reactions which did not provoke any immunity to subsequent challenge with T. parva. The next animal, No. 7359, also had a very mild disease but on recovery was found to be completely immune to challenge with T. parva. The parasite was then transmitted to Steer No. 7619 which suffered a severe reaction and died. The next four cattle, Nos. 7961, 7805, 7808 and 7834 all suffered a similar fate. The cattle of the 5th Passage, Nos. 8475 and 8536, both appeared to be infected with T. parva; one reacted and recovered and was immune on challenge with T. parva and the other reacted and died.

Steers Nos. 8253 and 8307 which had previously been immunized against T. parva by oral dosing with oxytetracycline hydrochloride (Brocklesby and Bailey, 1962) were completely refractory to infection. Similarly Steers Nos. 8480 and 8469 which were treated with the drug during their infection with "T. lawrencei (Kenya)", so that they recovered, were completely immune to challenge with T. parva.

According to the presently accepted diagnostic criteria, Cases 6590 and 7144 would be diagnosed as Tzaneen Disease (T. mutans infection), Case 7359 would be doubtful, possibly mild East Coast fever, and the subsequent cases would all be diagnosed as East Coast fever. But all these cattle were infected with the same parasite.

Passage Line B

Table XXIII shows the results of this passage line. It can be seen that this passage line showed no changes; it could be regarded as a serial passage of T. mutans since it consisted entirely of mild cases which conferred no immunity to subsequent challenge with T. parva.

Table XXIII

Summary of Cases in Passage Line B (see Fig. 11)

Animal No.	7148	7310	7618	8064	8447
No. of ticks	50	c.85	100	50	50
Incubation (days)	15	18	16	21	-
Reaction (days)	1	1	5	3	-
Max. Temp.	103.8	103.0	104.4	102.2	-
Result	R	R	R	R	R
Result of Challenge	D	D	D	D	D
Nuclei/ Schizont	5.15 (100)	6.6 (19)	-	7.41 (81)	-
Size of Schizonts (μ)	4.33 (100)	4.64 (19)	-	5.25 (81)	-
Max. piro. count	0	0	<1	2	9
Max.macro-schizont count	4	3	<1	5	<1
Max. micro-schizont count	0	0	0	0	0

Bracketed figures = the number of parasites examined or measured

D = Died

R = Reacted and Recovered

A NEW THEILERIAL PARASITE OF THE AFRICAN BUFFALO

History

The buffalo calf known as Steve was collected from the Mara District of Kenya when approximately 1 month old. He was very weak and had obviously recently been mauled by a predator, probably a lion or a leopard as he was covered with claw marks, which in most cases had led to abscess formation. The buffalo was treated with a daily low dose of oxytetracycline hydrochloride, injections of Vitamin B_{12} and occasional doses of magnesium sulphate and sulphadimidine. Work with oxytetracycline hydrochloride, in very high dosages for the treatment of T. parva infection, has shown that the drug does not affect the size or character of the schizonts.

For four days after the buffalo arrived at the laboratory thin blood films were negative. On the fifth day theilerial piroplasms were discovered (<1/1000 Red Blood Cells). The parasitaemia increased, the successive daily counts (per 1000 R.B.C.) being as follows:- <1, <1, <1, <1, <1, 1, 2, 18, 34, 46, 57, 38, 24, 19, 11, 10, 8, 8, 4, 6, 3, 4, <1, <1, 2, <1, <1. They were consistently present for the next 7 weeks when the buffalo was infected with T. parva.

On the 12th day a theilerial macroschizont was discovered in a thin blood film. These were present in blood films for 5 days but were always extremely rare (<1/1000 lymphocytes). Smears were prepared from the prescapular lymph nodes, which were not enlarged, for 11 days but in spite of lengthy observations no schizonts could be found.

Morphology of the Parasite

1. The Intra-erythrocytic Piroplasms.

These were typical theilerial piroplasms, being very pleomorphic with anaplasmoid, ring, comma and rod forms being frequent. Many forms suggesting multiplication were observed. Up to 6 parasites were seen in a single erythrocyte. Some of the forms seen are illustrated in Fig. 12.

2. The Schizonts.

After very long searches of numerous thin blood films a total of eighty-two schizonts was observed. They were mainly intra-lymphocytic but a few free parasites were seen. Most of the schizonts were obviously macroschizonts (Figs. 13 and 14) with rather large irregular pieces of chromatin. Two typical microschizonts were found (Fig. 15). However, some extraordinary schizonts were discovered which seemed to be a mixture of the two types (not intermediate forms). They contained many micromerozoites as well as cytomere-like blocks which were like macroschizonts (Fig. 16). I have never seen such parasites in cattle infected with T. parva.

The eighty macroschizonts were measured and their pieces of chromatin were counted. They were found to be very much larger than T. parva, T. mutans (Dr. S. F. Barnett, unpublished, found the average size of T. mutans schizonts to be 5.0μ) or T. lawrencei. Their average size was 13.1μ. The smallest schizont seen measured 5.6 x 4.8μ and the largest measured 36.4 x 21.2μ. The average number of nuclei per schizont was 27.3 with a range of 6-138. The comparable figures for T. parva were 8 nuclei/schizont, with a range of 1-85.

Figure 12

The Intraerythrocytic piroplasms of T. barnetti.
Several dividing forms are illustrated.

10 μ

Figure 13

Macroschizonts of T. barnetti. Note the large size of the parasites and the large irregular pieces of chromatin

ر١٥

Figure 14

Large macroschizont of *T. barnetti*.
The large irregular body in the centre is
a degenerate host cell nucleus.

15μ

Figure 15

A microschizont of T. barnetti
surrounded by erythrocytes

١٥ µ

Figure 16

A large "mixed" schizont of *T. barnetti* showing characteristics of macroschizonts and microschizonts

15μ

Attempts to Transmit the Parasite

Uninfected nymphae of R. appendiculatus were allowed to engorge on the buffalo at the time that piroplasms were frequent. They dropped fully engorged when the parasitaemia was 8 or 10 piroplasms/1000 R.B.C.: such a parasitaemia of T. parva would be more than adequate to infect this species of tick. After the ticks had moulted to adults they were allowed to feed on cattle and on a buffalo as follows:-

Fifty adult ticks were placed on each of two steers (Nos. 7188 and 7190). No reaction resulted and both animals were later challenged with T. parva and died.

Fifty adult ticks were allowed to feed on Buffalo Patrick but no infection resulted. However, this buffalo, collected as a calf from the Mara District of Kenya, had probably recently been naturally infected with the same parasite for, 6 days after his arrival at the laboratory, theilerial piroplasms appeared and slowly increased in number (up to 13/1000 R.B.C.). However, only one schizont was seen so that no close study could be made.

Attempts to Find the Parasite in Ticks

Eighty-nine of the adult R. appendiculatus, which had engorged on Buffalo Steve as nymphae, were sectioned, stained with Giemsa, and searched for parasites. Ten of the ticks were sectioned unfed but the remainder were allowed to feed on rabbits for varying periods of time. This procedure has been shown to be necessary for the demonstration of T. parva in sections of ticks (Martin, Barnett and Vidler, in press). Ten ticks were therefore allowed to feed on rabbits for 1, 2, 3, 4, 5, 6, and 7 days and nine ticks for 8 days.

No theilerial parasites could be found in the sections. This was very surprising since R. appendiculatus is an efficient vector of all the known bovine Theileria in East Africa.

Nomenclature and Provisional Definition

It is proposed to name this parasite Theileria barnetti n.sp. in honour of Dr. S. F. Barnett.

It is provisionally defined as follows:-

A theilerial parasite of the African buffalo. Schizogony takes place in lymphocytes of the peripheral circulation. Macroschizonts and microschizonts occur, together with schizonts that appear to be mixtures of the two types. Macroschizonts are large, averaging 13.1 μ in diameter and containing an average of 27.3 nuclei per schizont. After schizogony ceases piroplasms persist in the red cells for at least 7 weeks. The vector is not known.

DISCUSSION

All the experiments described in this account were carried out in Kenya and apply to conditions in East Africa. Although it may be tempting to suggest that similar phenomena may occur in Southern Africa where the African buffalo is found, this temptation should be resisted for the work of Neitz and his associates has not revealed anything comparable to the transformations seen in Kenya. However, the history of bovine theileriases in Southern Rhodesia affords an interesting parallel to some of the work described here and this will be referred to later.

The wild fauna is mainly responsible for the second industry of Kenya, the tourist industry. As this fact becomes more widely appreciated research work on the diseases of wild animals will be of increasing volume and importance. It is probable that game ranching schemes may be more productive, particularly in certain arid areas, than cattle ranches. The African buffalo, being a very large animal, may well have a part to play in such ventures. It is therefore of some importance to know something of the susceptibility of the buffalo to various diseases and the work described here has thrown a little light on this problem. It has been shown that the buffalo is strongly resistant to East Coast fever (T. parva infection): a total of 11 buffaloes have been exposed to the disease and not one of them has died. Only 2 of the buffaloes became sufficiently heavily infected to infect nymphal ticks that were feeding on them at the time. These ticks did, however, transmit fully virulent infections to cattle. This evidence indicates, then, that the buffalo, though very resistant to the disease, may play an important part in the maintenance of the causal parasite in areas where the common tick vector (R. appendiculatus) can survive.

Seven buffaloes have been infected with parasites thought to be strains of T. lawrencei and no less than 3 of them died. It is rather paradoxical that the buffalo should apparently be more susceptible to its own parasite than it is to infection with cattle strains of T. parva. However, the small numbers of buffaloes available for these studies does not permit a close analysis of the figures.

The significance of the results from the strictly veterinary point of view is the fact that it has conclusively been proved that the suspicion of farmers and veterinarians that the buffalo harboured an East Coast fever-like disease, is correct. Ticks, collected from an apparently healthy buffalo, transmitted a severe disease to cattle, causing a case mortality rate of 43%. The assumption is made that the parasite is normally maintained in buffalo herds and only occasionally enters cattle. The farmer must, therefore, take measures to ensure that the buffalo does not mix with his cattle: this can only be achieved by expensive fencing or by shooting the buffalo. It is suggested that there is no purpose, from the disease control standpoint, in differentiating between Corridor Disease and East Coast fever.

But the most interesting feature concerns the lability of "T. lawrencei (Kenya)". On its first isolation in cattle this organism conformed to the descriptions of T. lawrencei given by Neitz (1955, 1957). There was virtually no production of intra-erythrocytic piroplasms or microschizonts, and macro-schizonts were small and infrequent. The parasite was therefore diagnosed as T. lawrencei and has been referred to as "T. lawrencei (Kenya)" throughout this account. In spite of the fact that intra-erythrocytic piroplasms could not be found in thin blood films it was possible to transmit the parasite between cattle via the tick R. appendiculatus. This observation means that xenodiagnosis is the only reliable

technique to decide whether or not an animal is carrying theilerial piroplasms. This method requires the provision of a suitable vector tick and susceptible host animals. The subsequent passage of "T. lawrencei (Kenya)" through cattle resulted in remarkable changes. Copious production of piroplasms and microschizonts began to occur in several cases and by the time that the 6th passage was reached the majority of cases were indistinguishable from East Coast fever. These changes did not occur consistently in all the passage lines. From Tables XVII, XVIII, XIX and XX it can be seen that Passage Line 1 showed no change; Passage Line 2 underwent a sudden change between the 2nd and 3rd passage levels; Passage Line 3 consisted of cases that were difficult to diagnose and Passage Line 4 showed the changes with great clarity (Table XX); the first two cases (Nos. 4111 and 3975) would, according to the present differential diagnostic criteria, be diagnosed as T. lawrencei infection and the last 3 cases (Nos. 4618, 4352 and 5744) would certainly be diagnosed as T. parva infection.

A further example of the lability of "T. lawrencei (Kenya)" emerged from a study of the carrier status of recovered cattle. Neitz (1958a, 1958b) demonstrated that splenectomy of cattle recovered from infection with the South African strain of T. lawrencei resulted in a recrudescence of intra-erythrocytic piroplasms that were able to infect ticks. Seventeen intact cattle that had recovered from infection with "T. lawrencei (Kenya)" were tested for their carrier status by the feeding of nymphal ticks at varying intervals after recovery. Five of these cattle apparently became carriers. One of the cases (No. 6590) produced, by adult ticks that had engorged as nymphae on an animal that had recovered 8 months previously, was particularly interesting for it resulted in two passage lines in cattle that differed considerably (Tables XXI and XXII). One of these lines consisted of cases that changed from very mild infections, that

conferred no immunity to T. parva, into cases that were typical East Coast fever. The other passage line consisted entirely of cases that could only be diagnosed as being due to T. mutans.

Yet another example of theilerial lability, which is not described in the present account but which is worthwhile mentioning briefly in this discussion, has become apparent from the passage of T. parva (Icely) through cattle. This parasite was isolated by the employment of bait cattle in cattle-free buffalo-infested areas and must have originated in some wild animal, probably buffalo. It was briefly described as a "Mild Form of East Coast Fever" by Barnett and Brocklesby (1961). However, since this work was published the organism has increased in virulence and now produces typical East Coast fever, causing a high mortality rate in susceptible grade cattle even when small numbers of ticks are used to transmit it.

These observations show how changeable newly isolated strains of Theileria may be. There is no suggestion that the established laboratory strains of T. parva may be subject to such changes, though such a possibility should always be borne in mind. However, strains of T. parva, and "T. lawrencei (Kenya)"must certainly now be regarded as such, newly isolated from wild buffalo, may change considerably in character during early passages through cattle. This observation inadvertently casts doubt on the validity of T. lawrencei as a species, for the South African strain of the parasite has not been carried through many, if any, serial passages in cattle. Similarly a second strain isolated in Kenya (Barnett and Brocklesby, 1959), which was found by the exposure of cattle on buffalo grazings, was not infective for R. appendiculatus and so was not carried beyond the 1st passage in cattle. For the moment, however, T. lawrencei should still be regarded as a valid species, with the reservation that certain strains may be transmissible through cattle with ticks and that

such passages may be accompanied by alteration that may make them indistinguishable from T. parva. The parasite described here as "T. lawrencei (Kenya)" is in fact a strain of T. parva : the African buffalo in East Africa must therefore be regarded as a reservoir of East Coast fever.

The history of the disease in Southern Rhodesia indicates that something similar to the changes undergone by "T. lawrencei (Kenya)" may be taking place there. For many years the veterinary authorities in Southern Rhodesia evidently had little difficulty in differentiating East Coast fever from Theileriosis (T. lawrencei infection). The country has, in fact, been declared to be free of East Coast fever whereas the incidence of Theileriosis has increased (Table I). In later years there have been suggestions that Theileriosis was becoming increasingly difficult to differentiate from East Coast fever and Lawrence (1956a) reported that there appeared to be developing a closer resemblance between Theileriosis and East Coast fever than existed formerly. The presence of intra-erythrocytic piroplasms, generally rare but occasionally in considerable numbers, was observed and schizonts were sometimes as numerous as in East Coast fever (Lawrence, 1956b). It seems likely that the Southern Rhodesian Veterinary authorities have succeeded in eradicating the strain of T. parva introduced from Tanganyika only to be faced with a repetition of the natural history of the disease; its introduction from the wild African buffalo and gradual establishment in domestic cattle.

The extensive observations made on the morphology (size and numbers of nuclei) of this buffalo strain of T. parva have not been described in detail. It was hoped that, had the parasite been shown to be T. lawrencei, the figures would provide a basis for comparison with the figures for T. parva published by Barnett et al. (1961) and that a statistical analysis would lead to the establishment of reliable differential diagnostic criteria. However, the obvious transformations that the

parasite underwent rendered such a comparison purposeless.

The observations described recall the opinions expressed by Du Toit (1930). By referring to the earlier literature and to his own work he showed that the theilerial diseases of cattle could be arranged in eight categories of virulence. In this series, with highly virulent T. parva at one end and completely benign T. mutans at the other, the change from one extreme to the other was so gradual that Du Toit had the impression that

"we actually see before us species in the making".

He was obviously unhappy with the then accepted taxonomy of the theilerial parasites of cattle but did not then propose any changes. He pointed out that the difficulties could be overcome either by regarding all the parasites of cattle as varieties or strains of one species, T. parva, or by creating new species for each of the unnamed types of Theileria. The events described in the present account suggest that his first alternative may be correct for parasites like T. mutans and T. lawrencei have changed so that they become indistinguishable from T. parva. However, it is not proposed to introduce any changes in the nomenclature of these parasites here. The theilerial parasites of cattle that are acceptable as good species are T. parva, T. annulata, T. mutans and, with the reservations mentioned, T. lawrencei.

In 1952, Dschunkowsky prefaced an article on the theilerioses with the following remark:-

> "The group of diseases discovered at the beginning of this century, and known as theilerioses, is still very much of a riddle, and much regarding it is still open to further investigation".

The work outlined here does not go very far towards a solution of the riddle but certainly emphasises what a profound and complicated riddle it is.

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[Note: Many of the references are from the various Annual Reports of the Veterinary authorities of Southern Rhodesia. Since the arrangement of these reports is rather confusing it may clarify matters if this is briefly described. We are only concerned with reports for the year 1933 onwards. The reference date of each report is usually, but not invariably, the year following that to which the report refers. Reported years sometimes ended on 31st December and sometimes on some other date such as 30th September. Until the report dated 1949 (for 1948) the reports are in two separate parts:

1. Report of the Chief Veterinary Surgeon for the Year ....
2. Report of the Director of Veterinary Research for the Year ....

With the report dated 1949 (for 1948) the two parts became fused, in one binding, under the title "Report of the Director of Veterinary Services for the Year 1948"; this was in two parts:

1. Report of the Chief Veterinary Surgeon for the Year 1948
2. Annual Report of the Assistant Director of Veterinary Services (Research) for the Year 1948.

In subsequent years Part 1 is entitled "Report of the Director of Veterinary Services for the year .....".

In 1952 a third part was interposed between the other two so that the "Report of the Director of Veterinary Services" then contained three parts:

1. Report of the Director of Veterinary Services for the Year 1951.

2. Annual Report of the Assistant Director of Veterinary Services (Field) for the year ending 30th Sept., 1951.

3. Annual Report of the Assistant Director of Veterinary Services (Research) for the year ending 30th Sept., 1951.

With the report dated 1955 (for 1954) came another change due to the formation of the Central African Federation. This report was entitled "Federation of Rhodesia and Nyasaland. Annual Report of the Secretary to the Federal Ministry of Agriculture which includes The Department of Research and Specialist Services, Conservation and Extension Services and Veterinary Services for the year ended 30th September, 1954". This system has been continued and this large report always includes the section on Veterinary Services which is usually divided into the familiar three parts:

1. Report of the Director of Veterinary Services.

2. Report of the Assistant Director of Veterinary Services (Field).

3. Report of the Assistant Director of Veterinary Services (Research)]

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